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Synthetic and Alternate Fuels Characterization

Final Report

February 1, 1988

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Diesel fuels derived from petroleum and synthetic sources were compared chemically and toxicologically to address the issue of whether a changeover of mobility fuels from natural to synthetic sources might be accompanied by greater or different toxicological hazards to military personnel. Diesel fuels derived from petroleum, shale oil, tar sands, and tar sands/petroleum coprocessing were compared for major organic composition, 4-6 ring polycyclic aromatic hydrocarbon dermal tumorigens, major organic compounds in their inhalable volatiles, mouse dermal tumorigenicity and promoting activity. The database was expanded by a similar comparison of petroleum- and coal-derived fuels for the U.S. Department of Energy (USDOE). The results suggest that highly refined and finished mobility fuels from synthetic or alternate sources will not pose a significantly greater toxicological hazard than current petroleum-based fuels. Toxicological differences are likely to be subtle.

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SYNTHETIC AND ALTERNATE FUELS CHARACTERIZATION

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EXECUTIVE SUMMARY

The Department of Defense is concerned with determining if a changeover from petroleum- to shale oil-derived or other synthetic mobility fuels would be accompanied by a significantly greater or different toxicological hazard to military personnel who are exposed to the fuels in their military occupations. Dermal and inhalation toxicology are the primary concerns, and tumorigenesis is the main biological endpoint considered. A set of diesel fuels (DF) representing petroleum, shale oil, tar sands, and tar sands/petroleum coprocessing technologies were compared chemically and toxicologically. The comparative characterization included determinations of physical and chemical properties, the major organic chemical composition of the liquid fuels and their inhalable vapors, and the benzene, alkyl benzene, and 4- to 6-ring polycyclic aromatic hydrocarbon dermal tumorigen content of the liquid fuels. The comparative toxicology consisted of mouse skin-painting bioassays of the tumor promoting activity and complete tumorigenicity using the SENCAR mouse strain. The available database was expanded by a U.S. Department of Energy Office of Fossil Energy (DOE/FE) sponsored study comparing the toxicity of fuels refined from coal liquids and petroleum. Many of the same experimental protocols were used in that study.

The liquid fuels were found to be qualitatively similar in their major organic chemical composition, and the compositional differences were mainly quantitative. These differences appeared to be generic between petroleum- and shale oil-derived DF. The shale oil-derived DF were lowest in aromatics, followed by the petroleum-derived DF, and finally the experimental tar sands/petroleum coprocessing DF was the highest in aromatics content. Similar trends were found for the composition of the inhalable vapors. All the fuels were found to exhibit tumor promoting and complete tumorigenic activity. There were some differences in tumor response between male and female mice. The tar sands/petroleum coprocessing DF was notably high in both tumor promoting activity and complete tumorigenicity with both sexes. The complete tumorigenicity of this fuel appeared to correlate with its relatively high concentrations of PAH which are believed to be contributed by the petroleum-derived light cycle oil blended into the fuel. The petroleum-derived DOD Referee DF-2 was close to the tar sands/petroleum coprocessing fuel in tumor promoting activity, while the shale oil-derived DF and tar sands-derived railway DF were lowest in promoting activity with female and male mice (respectively). For complete tumorigenicity, the petroleum-derived DOD Referee DF-2 and the Petroleum Reference DF-2 were next in potency with female and male mice (respectively), and the shale oil-derived DF-2 and tar sands-derived railway DF were lowest in complete tumorigenicity with female and male mice (respectively). The relative order of tumor promoting activity and complete tumorigenicity was the same for a given sex, suggesting the importance of promotion to the expression of PAH tumorigenicity.

The results of this study suggest that (with the possible exception of the experimental tar sands/petroleum coprocessing DF) highly refined, synthetically-derived mobility fuels will not pose unusual toxicological risks compared to their petroleum counterparts. Rather, differences in toxicity are likely to be subtle.

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INTRODUCTION

Mobility fuel availability is critical to the security of the United States. However, ca. 25 percent of the crude oil needs of the United States are met by foreign imports which may be depleted early in the next century and which are highly vulnerable to interruption by political or armed conflicts. The development of domestic synthetic and alternate sources of feedstocks and their production into mobility fuels is of considerable strategic importance.

The U.S. Army has the lead role in the development of the capability to utilize diesel fuel (DF) derived from synthetic and alternate sources, while the Navy and Air Force have lead roles in aviation gasoline and diesel fuel marine. Shale oil is considered as a primary candidate for the production of DF, and the original plans for the Army were to evaluate the behavior and vehicle performance of a large production run of shale oil-derived DF at two installations. Unfortunately, the failure of another Department of Defense contractor to produce sufficient crude shale oil for refining into DF, plus the current surplus of crude oil supplies have delayed the accomplishment of this plan.

Among the primary health-related concerns of the Army are the potential toxicological hazards to military personnel from the handling and use of synthetically-derived fuels versus current petroleum-derived fuels. Mouse skin-painting bioassays (1-3) have demonstrated that crude shale oil and crude coal liquids are considerably more tumorigenic than petroleum crude oils. These synthetic crude oils also are chemically different from crude petroleum, but compositional differences decrease with increased refining (4,5). It is not known if the exposure of military personnel to the vapors and liquids of synthetically-derived fuels could result in a greater or different type of toxicological hazard relative to that posed by current petroleum analogs. This project addressed that question as regards DF. The routes of exposure considered were inhalation and dermal contact, and the main toxicological endpoint of concern was tumorigenicity. Although the primary focus was on DF derived from petroleum and shale oil, additional synthetic sources of DF, including tar sands and tar sands/petroleum coprocessing, were included. This report describes the comparative characterization of the physical and chemical properties, and liquid and inhalable vapor organic compositions of these fuels, and of their complete tumorigenicity and tumor promoting activity. The database has been expanded considerably by a toxicological comparison of coal liquids and petroleum-derived fuels sponsored by the U.S. Department of Energy, Office of Fossil Energy. Many of the experimental protocols were the same in both studies.

The results of this comparative chemical and toxicological characterization of the synthetic- and petroleum-derived fuels are reported in this document. Related concerns regarding end-product use and military personnel exposure to fuel-related contamination of the workplace atmosphere are addressed in a companion project, "Field Sampling and Analysis of Shale Oil Derived Airborne Diesel Exhaust," Army Project Order No. 84PP4867. The results of that study are being reported separately.

FUEL SOURCES AND COMPARATIVE PROPERTIES

Sources

The fuels chosen for study in this project, their sources, and the rationale for their selection are described below. They consisted of five diesel fuels derived from both petroleum and synthetic origins. The two petroleum-derived fuels were selected to serve as "benchmarks" for comparison with the synthetically-derived fuels. These petroleum-derived fuels represent the diesel fuel compositions to which military personnel are currently exposed. These fuels are available from commercial sources. The latter three fuels represent synthetic mobility fuel technologies which might be utilized in a national emergency to supplement petroleum fuels which are heavily dependent upon foreign crude oil sources. Only one of these synthetic fuels is commercially available.

Petroleum-Derived Fuels

Two petroleum-derived fuels were included in the study to serve as points of comparison with the synthetically-derived fuels. They consisted of the following:

Phillips Petroleum Reference DF-2: This fuel is a commercially available petroleum reference DF-2 which is marketed for testing purposes requiring good lot-to-lot reproducibility in composition and properties. It is used by the U.S. Environmental Protection Agency (USEPA) for diesel engine emission certification and mileage testing (6). This fuel was selected to represent high quality petroleum-derived diesel fuels. Lot no. C-345 of this fuel was used in earlier studies of fuel toxicology and chemistry (7-9) for the U.S. Army Biomedical Research and Development Laboratory (USABRDL).

Two 209 L (55 gallons) drums of lot no. C-747 of this fuel (catalog no. RF-2844) were purchased from the Phillips Chemical Company (Specialty Chemicals, Drawer O, Borger, TX 79007) and were received on 11/3/82. Two additional drums of the same lot no. were received on 6/10/83. These four drums were assigned the sample numbers 1910-1913 by the DOE Synthetic Fuels Repository at ORNL. They were stored at 3°C in a secure, temperature-monitored cold storage facility. Sample no. 1910, which was used for the chemical and toxicological characterization, was from the first shipment. To promote stability, it was mixed by rotation for 5 min on a barrel rotator, transferred into a type 314 stainless steel drum, and the drum headspace was briefly flushed with argon before sealing. At the time of transfer, aliquots for chemical and toxicological characterization were taken into amber borosilicate bottles and the headspace of each bottle was briefly flushed with argon before the bottles were capped with Teflon-lined screwcaps. These aliquots were stored at 3°C in a flammables-rated refrigerator. Properties for lot no. C-747 of Phillips Reference DF-2 are listed in Table 1.

Table 1. Specifications and Properties of Diesel Fuels Derived from Petroleum and Synthetic Fuels

Property	Petroleum: Phillips Reference 1910[a]	Petroleum: DOD Referge 1914[b]	Shale Oil: Geokinetics/ Suntech 4801[c]	Tar Sands: Suncor Railway 9527[d]	Tar Sands/Petrol.: Canadian 1990 9523[c]	DF-2 CONUS[e]
Specific Gravity	0.8463[f]	-	0.8275[f]	0.8757-0.2494[f]	0.8899	-
Gravity, °API	35.7	-	39.5	30-35	27.5	-
Cetane Number	47.1	40-45	51.1	-	34.9	45 min.
Carbon Residue on 10% Bottoms, Wt.%	-	0.20 max.	-	-	-	0.35 max.
Distillation, Range, °C						
IBP	189	-	180	-	170	-
5%	206	-	197	216 max.	191	-
10%	215	-	207	-	392	-
50%	261	245-285	251	271 max.	517	-
90%	300	330-357	304	-	667	338 max.
95%	310	350-375	320	343 max.	700	-
EP	324	385 max.	341	-	763	370 max.
Residue, Vol. %	1	-	1.0	-	1	3 max.
Kinematic Viscosity, cSt @ 40°C	2.40	1.9-4.1	2.44	-	2.91	1.9-4.1
SUS @ 38°C	-	-	-	30-40	-	
Flash Point, °C	69[g]		69	-	62	52 min.
Pour Point, °C	-18	-18 max.	-18	-40 max.	-42	-
Cloud Point, °C	-19	-13 max.	-	-	-	-
Particulate Matter, mg/L	2.39	10 max.	-	-	-	10 max.
Accelerated Stability, Insolubles, mg/100 mL	3.3	1.5 max.	4.8	-	30.1	1.5 max.
Copper Strip Corrosion,[h] ASTM	-	1 max.	-	-	-	3 max.
Reid Vapor Pressure, psi	0	-	0	-	-	-
Calculated Vapor Pressure, PSI (500°F)	26	-	34	-	-	-

[a]Data supplied for lot C-747 by Southwest Research Institute (ref. no. 6).
[b]Military specification MIL-F-46162B
[c]Data supplied by Southwest Research Institute (ref. no. 13).
[d]Quality Ranges supplied by Suncor, Inc.
[e]Federal specification VV-F-800 C
[f]16.7°C
[g]Data supplied for Lot C-345 by Phillips Chemical Co.
[h]3 hrs @ 50°C

Table 1. Specifications and Properties of Diesel Fuels Derived from Petroleum and Synthetic Fuels

Property	Petroleum		Shale Oil	Tar Sands	Tar Sands/Petrol.	
	Phillips Reference 1910[a]	DOD Referee 1914[b]	Geokinetics/ Suntech 4801[c]	Suncor Railway 9527[d]	Canadian 1990 9523[c]	DF-2 CONUS[e]
Heat of Combustion, MJ/kg						
gross	45.363	-	45.996	-	44.208	-
net	42.614	-	43.068	-	41.715	-
Ultimate Analysis, Wt.%						
C	86.69	-	86.0	-	87.04	-
H	12.96	-	13.8	-	11.75	-
N	0.004	-	0.094	0.035 max.	0.028	-
S	0.20	0.95-1.05	0.04	0.2 max.	0.67	0.50 max.
O	0.045	-	0.060	-	-	-
Ash, Wt.%		0.02 max.	-	-	-	0.01 max.
Hydrocarbon Type (FIA), vol.%						
Saturates	70.8	-	81.0	-	32.7	-
Olefins	1.2	-	1.2	-	0	-
Aromatics	28.0	-	17.8	60 max.	67.3	-
Aromatic Carbon, Wt.%						
Monocyclic	5.86	-	4.12	-	10.65	-
Dicyclic	9.79	-	1.53	-	10.18	-
Tricyclic	1.01	-	0.21	-	3.31	-
Neutralization No., TAN	-	0.2 max.	-	-	-	-
Additives, ptb	10.0[i]	-	-	-	-	-

[i] DuPont FOA #11 (Data supplied for Lot C-345 by Phillips Chemical Co.).

DOD Referee Grade DF-2: This high sulfur content petroleum DF-2, MIL-F-46162B, was included to represent a "worst case" fuel which barely meets military specifications, such as would be produced during a national emergency. The USABRDL Project Officer arranged through Mr. Maurice E. Lepera, Chief of the Fuels and Lubricants Division, Materials, Fuels and Lubricants Laboratory, U.S. Army Belvoir Research & Development Center, Fort Belvoir, VA, for one 209 L (55 gallons) drum to be shipped to ORNL from the US Army Tank-Automotive Command, Warren, MI. One drum labeled as "High Sulfur Fuel, FSN No. 9140-NSR, Mfg. No. 46H06-3322-0408" was received on 12/13/83. It was assigned sample no. 1914, and was stored at 3°C in the original drum. Aliquots for study were taken as described above.

The military specifications MIL-F-46162B for this fuel are included in Table 1.

Additional Petroleum-Derived DF-2: Additional samples of petroleum-derived DF-2 were used in the comparative chemical characterization to extend the chemical database and allow an assessment of the variability among a given fuel type. These fuels consisted of no. 9101 Phillips Chemical Co. Referee DF-2, lot no. C-345 (used in a previous study for the USABRDL, references 7 and 8), no. 4616 petroleum diesel fuel marine (DFM) used in the petroleum- and Paraho shale oil-derived fuels toxicology study (10) by the U.S. Navy Toxicology Detachment at Wright-Patterson Air Force Base (WPAFB), OH, and samples DF-2-1 through DF-2-3 which were collected at the DIO motor pool, 4/68 Armored motor pool, and 4th Engineers motor pools (respectively) at Fort Carson, CO during a diesel engine exhaust workplace air sampling trip in 9/84.

Shale Oil-Derived Fuel

Oil shale and coal are the two main sources available for production of synthetic fuels. Shale oil is a more desirable synthetic source for diesel fuel production because it contains a much greater proportion of aliphatic compounds than do crude coal-derived liquids. Accordingly, a shale oil-derived DF was included in this study. Samples of shale oil-derived DF-2 were obtained from Suntech, Inc., Marcus Hook, PA, through Mr. Norman R. Sefer, Senior Research Engineer, Southwest Research Institute, San Antonio, TX. Dr. Ralph D. Fleming of the U.S. DOE Office of Vehicle and Engine R&D, Conservation and Renewal Energy, Office of Fossil Energy, advised us of these fuels and made them available to us. They are derived from a 1981 in-situ production of shale oil by Geokinetics at Vernal, UT. The crude shale oil was subjected to "moderate severity" hydrotreating by Hydrocarbon Research, Inc., at the Lawrenceville, NJ facility and was distilled by Suntech at Marcus Hook, PA.

Two 209 L (55 gallons) drums of shale oil-derived DF-2 were received at ORNL on 3/3/84. One drum of DF contained an antioxidant while the second lacked this additive. They were assigned sample nos. 4802 and 4801 (respectively). The second drum (no. 4801, DF-2 without antioxidant), which was used in this study, was received with a tag labeled "Drum No. P10-848, No. 2 Diesel from Shale Oil, No Antioxidant". Both fuels were transferred to type 314 strainless steel drums. Aliquots for study were taken and the fuels were stored as noted above. Fuel properties are listed in Table 1.

An additional sample of shale oil-derived DFM no. 4610 was included in the comparative chemical characterization studies. This was the Paraho shale oil-derived DFM refined by SOHIO (11) for DOD toxicology and combustion studies. It was included in the comparative petroleum/shale oil fuels toxicology study (10) conducted at Wright-Patterson Air Force Base, OH by the U.S. Navy Toxicology Detachment.

Tar Sands and Tar Sands/Petroleum Coprocessing-Derived Fuels

Tar sands also are a viable synthetic crude oil source for DF production. Much progress in producing useful fuels from tar sands is being made in Canada. The two tar sands-derived fuels used in this study represent two approaches to the production of DF. One is a 100 percent tar sands-derived fuel which already is at the commercial stage, while the second is an experimental fuel from the coprocessing of tar sands and petroleum crude oil.

Suncor Railway DF: This is a commercially available DF which is sold by Suncor, Inc., Calgary, Alberta, Canada to the Canadian railroads as a DF. It is derived (12) from Alberta tar sands by hot water extraction, dilution and filtration, and coking of the bitumen after removal of the diluent. The liquids from the coking are distilled into naphtha, kerosene, gas oil, and a gas oil sidestream. The latter is sold as upgraded DF to railroads. One 209 L (55 gallons) drum of each product was received on 4/8/85. The railway DF was tagged "Mar 25/85, 95X29766V [this is the ORNL purchase requisition no.], RTS 2181." It was assigned sample no. 9527 and was stored as described above. Fuel "quality ranges" data supplied by Suncor, Inc., are listed in Table 1.

1990 DF: This DF is derived from the coprocessing of tar sands crude oil and petroleum crude oil. This experimental fuel is intended to represent a "typical" DF from the 1990s when tar sands crude oils are expected to compose ca. 25 percent of the feedstock of Canadian petroleum refineries. It is described (13) as being composed of 78 vol percent of a diesel cut from the refining of a 50/50 mixture of tar sands synthetic crude oil and conventional Alberta crude oil and 22 vol percent of hydrotreated cut-cracked cycle oil (petroleum) from another refinery. Dr. Ralph Fleming of the U.S. DOE obtained this fuel for us through Dr. Robert B. Whyte, Head of the Fuels and Lubricants Laboratory, National Research Council of Canada, Ottawa,

Ontario, Canada. One 209 L (55 gallon) drum of the 1990 DF, labeled "1990 FLO 8224C," was received on 1/14/85. It was assigned sample no. 9523 and was stored as described above. Sample properties are included in Table 1.

Parallel DOE/FE Study of Coal- and Petroleum-Derived Fuel Oils and Naphthas

The database available to both the DOD and DOE/FE is expanded considerably by the use of identical protocols for parts of both studies, in particular, the mouse skin painting bioassays. In the same time frame as for the DOD studies, four additional fuels were examined for the U.S. Department of Energy, Office of Fossil Energy (DOE/FE). These included two coal-derived fuels and two additional petroleum-derived fuels. They are described in detail elsewhere (5). A brief description of these fuels is given below.

H-Coal Home Heating Oil: This fuel was prepared to represent a coal-derived fuel suitable for home heating purposes such as is no. 2 fuel oil. It was derived from a 40/60 (wt/wt) blend of H-Coal light and heavy oils from the Catlettsburg, KY pilot plant run no. 8 on Illinois No. 6 coal in the Synfuel mode. The blending and subsequent high severity hydrotreating (3,000 SCF hydrogen/barrel) were performed by the Chevron Research Company (Richmond, CA). Devolatilization to meet the ASTM flash point specification for no. 2 fuel oil was conducted at ORNL. This fuel was assigned sample no. 978.

API No. 2 Fuel Oil: This petroleum-derived fuel (API product no. 83-02) was supplied by the American Petroleum Institute (API, Washington, DC). It was selected by the API as a typical no. 2 fuel oil against which to compare the coal-derived home heating oil. Documentation supplied with the fuel by the API describes it as 70 percent straight run middle distillate (straight run diesel [VPS #5 stripper, 82-3808]) plus 30 percent light catalytically cracked distillate (FC light cycle oil gas oil, 82-3843). It was assigned sample no. 975.

H-Coal Reformed Naphtha: This fuel was prepared from the same H-Coal light/heavy oil blend as was the H-Coal Home Heating Oil. Chevron performed a high severity hydrotreatment followed by hydrocracking. Universal Oil Products, Inc. (now the Signal Research Center, Inc., Des Plaines, IL), conducted catalytic reforming to yield a 96 octane gasoline product. It was assigned sample no. 936.

API Light Catalytically Cracked Naphtha: This petroleum-derived gasoline product (API product no. 81-04) was supplied by the API as a benchmark for comparison with the coal-derived gasoline product. It was described by the API as being produced by distillation of products from a catalytic cracking process. It was assigned sample no. 976.

Comparison of Properties

Comparison of the available property and specification data for the five fuels listed in Table 1 suggests that the 1990 tar sands/petroleum coprocessing DF (sample no. 9523) and the Geokinetics/Suntech shale oil-derived DF (no. 4801) represent opposite extremes bracketing the properties of the two petroleum- and one tar sands-derived DF. The experimental 1990 DF is characterized by relatively high density, viscosity, boiling range, aromatics, and S content, and the lowest cetane no. and accelerated stability test result. Most of these factors are interrelated. For example, the extended upper boiling range and total aromatics content are associated with its much greater percentages of di- and triaromatics. The very high final boiling point indicates that this fuel contains a significantly greater proportion of relatively low volatility matter than the other fuels. This high-boiling matter includes the four- to six-ring polycyclic aromatic hydrocarbon (PAH) dermal tumorigens, which also were determined in these fuels (see later sections of this report). Discussions with staff of the Canadian National Research Council indicated that the aromatic compounds were contributed largely by the petroleum-derived light cycle oil which was blended with the tar sands/petroleum component.

Several of the properties of the experimental 1990 DF would not meet the federal specification VV-F800C for DF-2 used in the continental US (CONUS). These properties include the 90% volume distillation and end point of the distillation range, accelerated stability test, and total S content. It is likely that these properties could be improved if the blending ratio of the light cycle oil is reduced.

In contrast, the shale oil-derived DF (no. 4801) was the least dense, contained the least aromatics and total S, and was the highest in saturates, cetane no., and total H. The only federal DF-2 specification it would not meet is the accelerated stability test, which is intended only for tactical, OCONUS (outside of the continental US), or long term storage (greater than 6 months) applications. Otherwise, it appears to be an excellent grade of fuel.

The Phillips Reference DF-2 (no. 1910) also is a high quality fuel which meets all specifications except for the accelerated stability test. It is intermediate between the 1990 DF and the shale oil-derived DF-2 in many of its properties. The minimum-specification DOD Referee DF-2 (no. 1914) is notably high in total S content.

TOXICOLOGICAL COMPARISON OF FUELS

The two petroleum- and three synthetically-derived DF were compared for their tumor promotion and complete tumorigenic activities in a mouse dermal assay. Previous studies for the DOE/FE suggested (5) that tumor promotion is important to the complete tumorigenicity of highly refined fuels derived from coal liquids and petroleum. It was observed in that study that the complete tumorigenicity of the four fuels (briefly described in the last section) did not correlate with their contents of known tumor initiators such as certain four- to six-ring PAH. The two fuels exhibiting the highest (H-Coal Home Heating Oil, no. 978) and least (H-Coal Reformed Naphtha, no. 936) tumorigenicity with the C3H mouse strain were found to contain nearly the same concentrations of these PAH, which were orders of magnitude greater than in the other two (petroleum-derived) fuels. The latter exhibited intermediate levels of tumorigenicity. The hypothesis that tumor promotion is important to the complete tumorigenicity of these refined fuels was investigated in a subsequent study using the C3H and SENCAR strains.

The toxicological comparison of DF for the DOD also utilized the SENCAR mouse strain because its high sensitivity to tumorigens allows a good resolution of tumorigenicity in a much shorter time frame than with less sensitive strains such as the C3H. A single dose level protocol for comparing tumor promoting activity among the fuels was used to allow maximum sensitivity and economy. An important feature of this protocol is that a comparison of the complete tumorigenicity of the fuels was obtained in the control groups lacking the tumor initiator dose. Use of the same protocol as for the DOE/FE study and in the same time frame greatly expanded the database available to each agency.

Toxicology Protocol

The protocol for this study was ORNL Biology Division study plan no. 10-09-85. The same protocol was used for the samples in the DOE/FE study. Details of the protocol are given below.

Source: The SENCAR mice were obtained from the Oak Ridge Research Institute (Oak Ridge, TN), and were 8 to 12 weeks of age at the time of first treatment.

Husbandry: The animals were grouped five to a cage in plastic "shoe-box" cages. They were fed a commercial laboratory diet (Ralston Purina Rodent Chow 5001) and tap water (16 ppm chlorine, 2 ppm fluoride) ad libitum, and were exposed to a daily light/dark cycle of 12 hrs each continuous light and darkness. The rooms were environmentally controlled to maintain a temperature of 18-26°C and a humidity of 40-60 percent.

Experimental Groups: At the end of a 4-5 week acclimation period, the animals were randomly assigned to experimental groups of 25 males and 25 females each. The experimental groups received the following treatments:

Tumor Promotion Activity-

DMBA then No. 1910
DMBA then No. 1914
DMBA then No. 4801
DMBA then No. 9523
DMBA then No. 9527

Fuel Controls (Complete Tumorgenic Activity)-

Acetone then No. 1910
Acetone then No. 1914
Acetone then No. 4801
Acetone then No. 9523
Acetone then No. 9527

Positive Control-

DMBA then TPA

Negative Controls-

DMBA then Acetone
Acetone then TPA

Dose and Application Schedule: The mice were treated with either 200 μL of acetone or acetone containing 2.52 μg of 7,12-dimethylbenz-[a]anthracene (DMBA) two days after being shaved with electric clippers. Seven days later, twice-weekly treatments were begun with 200 μL of the neat fuel (100% concentration), acetone, or acetone containing 2 μg of 12-O-tetradecanoylphorbol-13-acetate (TPA).

Observations and Termination: Treatments continued for 52 weeks. The animals were examined weekly for tumors and general health. The number of tumors was recorded. Those animals surviving for 52 weeks were terminated by carbon dioxide inhalation.

Comparative Toxicology of Diesel Fuels

Figures 1-5 are plots of the cumulative tumor incidence as a function of treatment time for the tumor promotion and complete tumorigenicity

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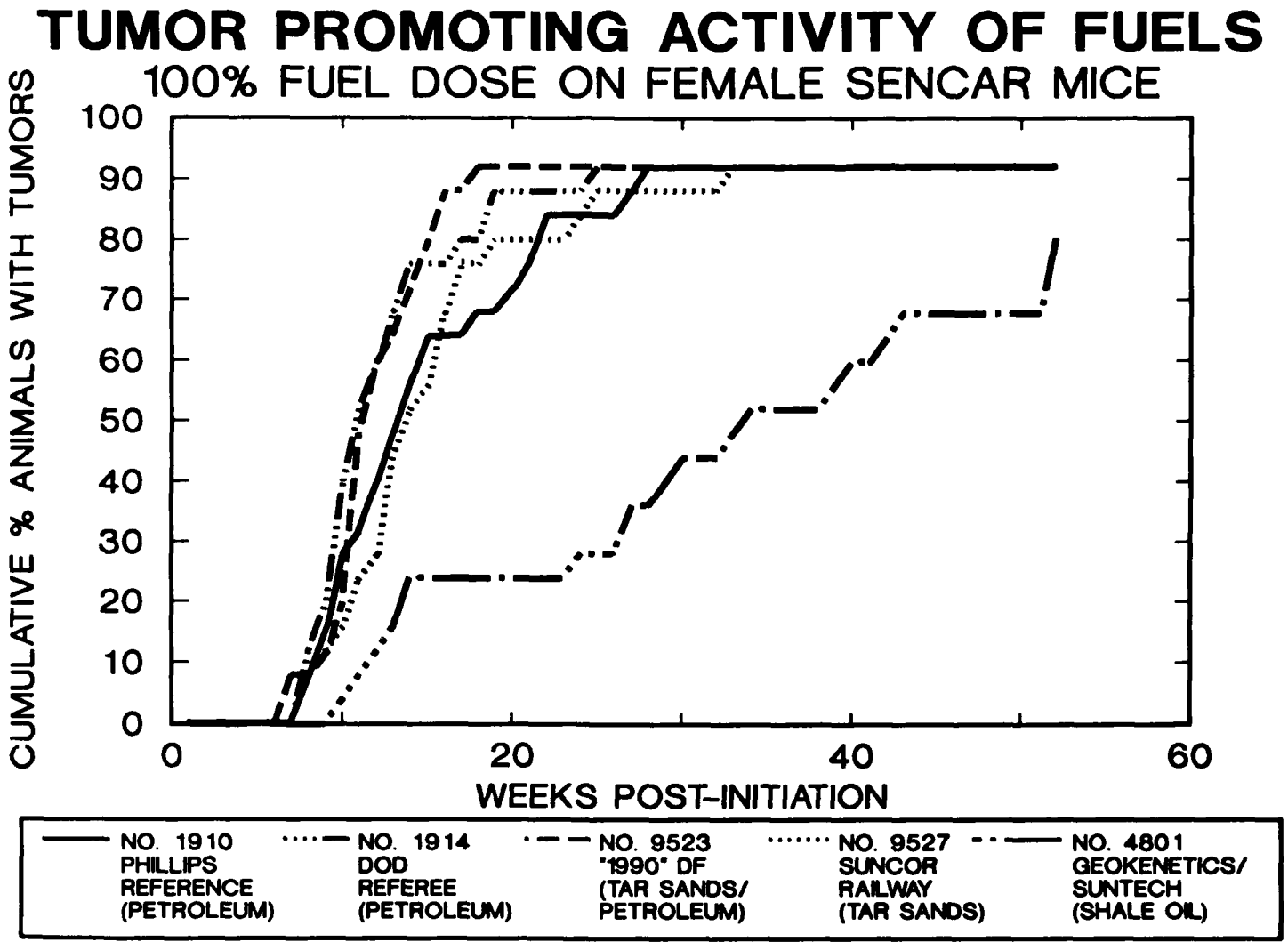

Figure 1. Cumulative Tumor Incidence (Female Sencar Mice) in the Comparative Tumor Promotion Bioassay of Diesel Fuels Derived from Petroleum and Synthetic Sources

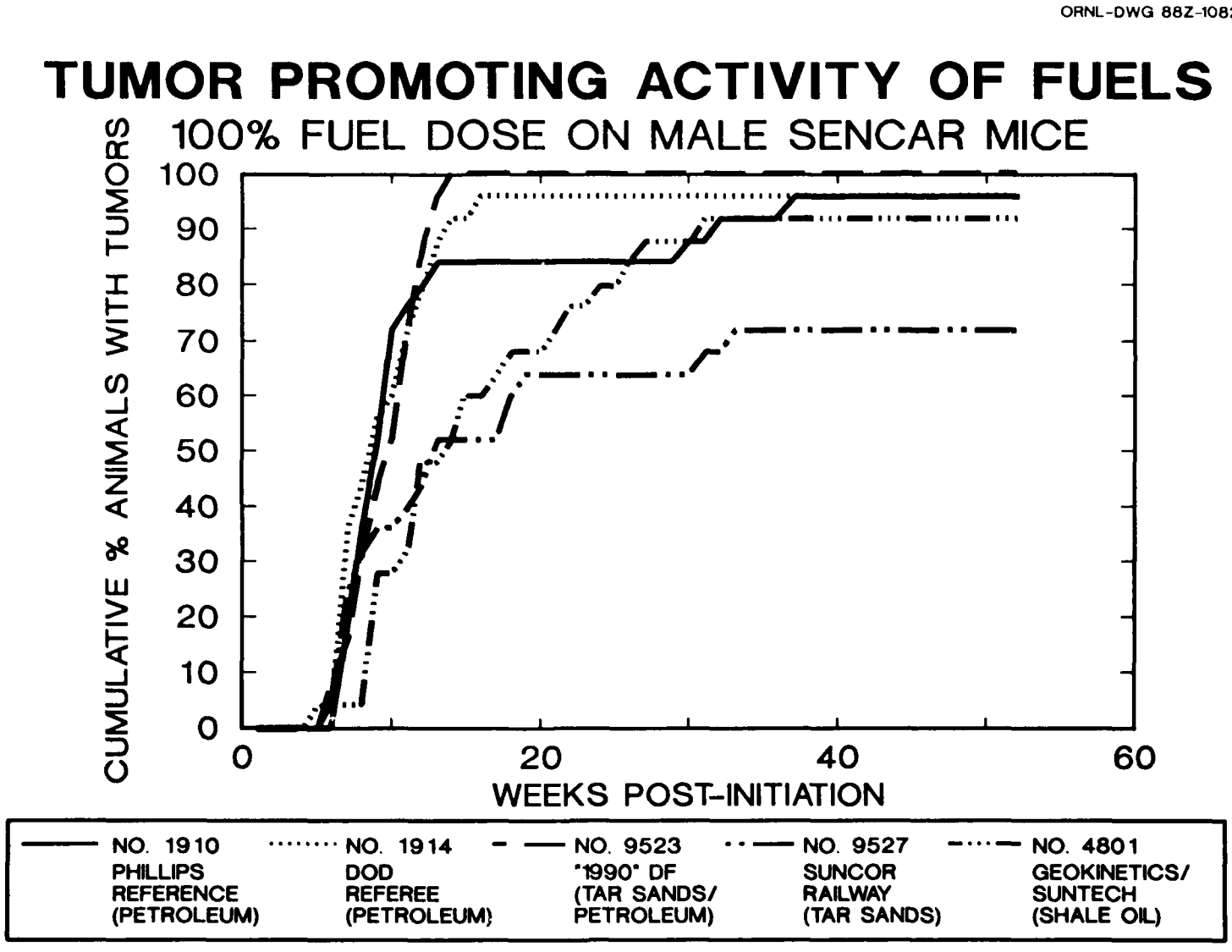

Figure 2. Cumulative Tumor Incidence (Male Sencar Mice) in the Comparative Tumor Promotion Bioassay of Diesel Fuels Derived from Petroleum and Synthetic Sources

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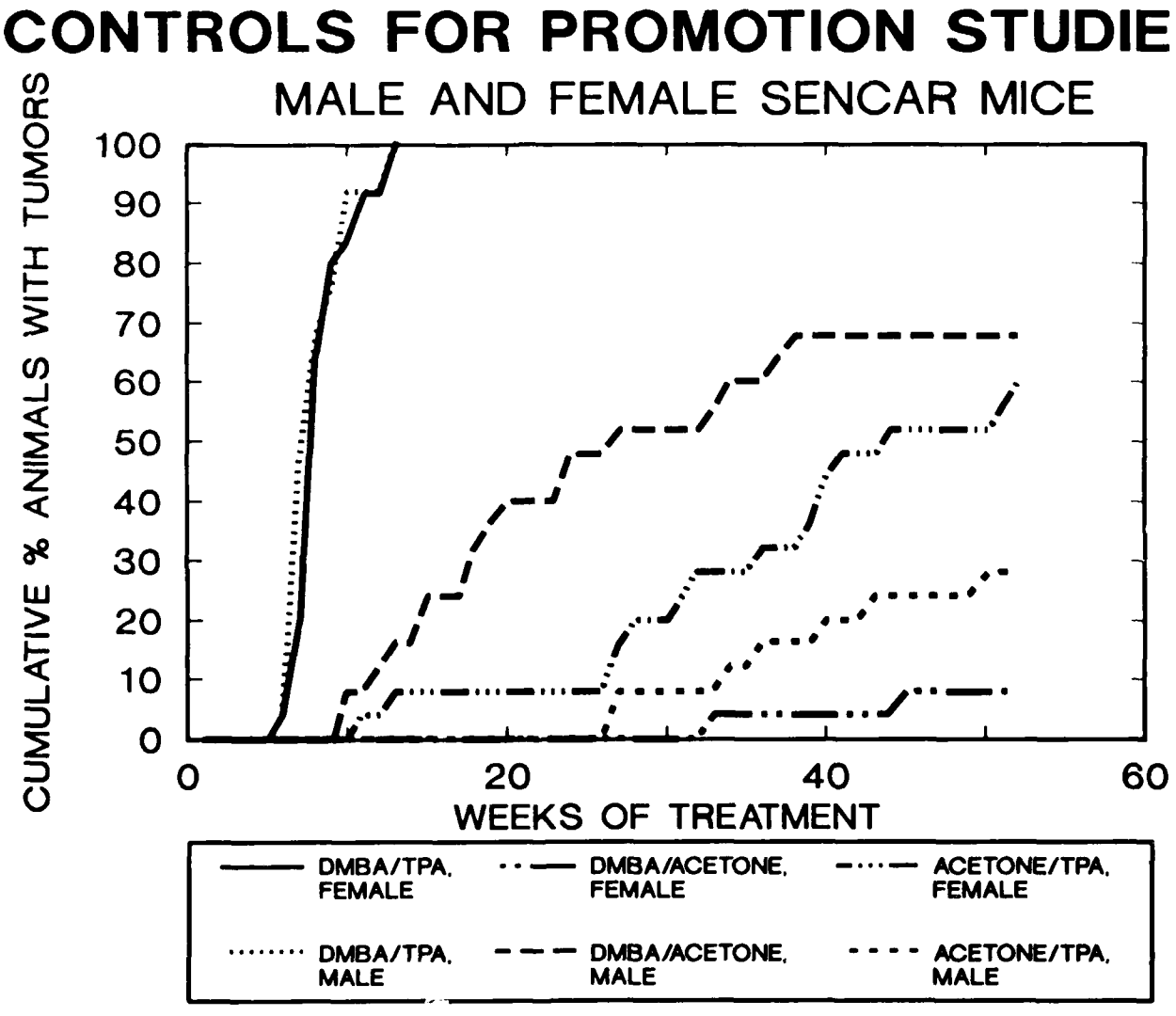

Figure 3. Cumulative Tumor Incidence for the Control Groups in the Tumor Promotion Bioassay

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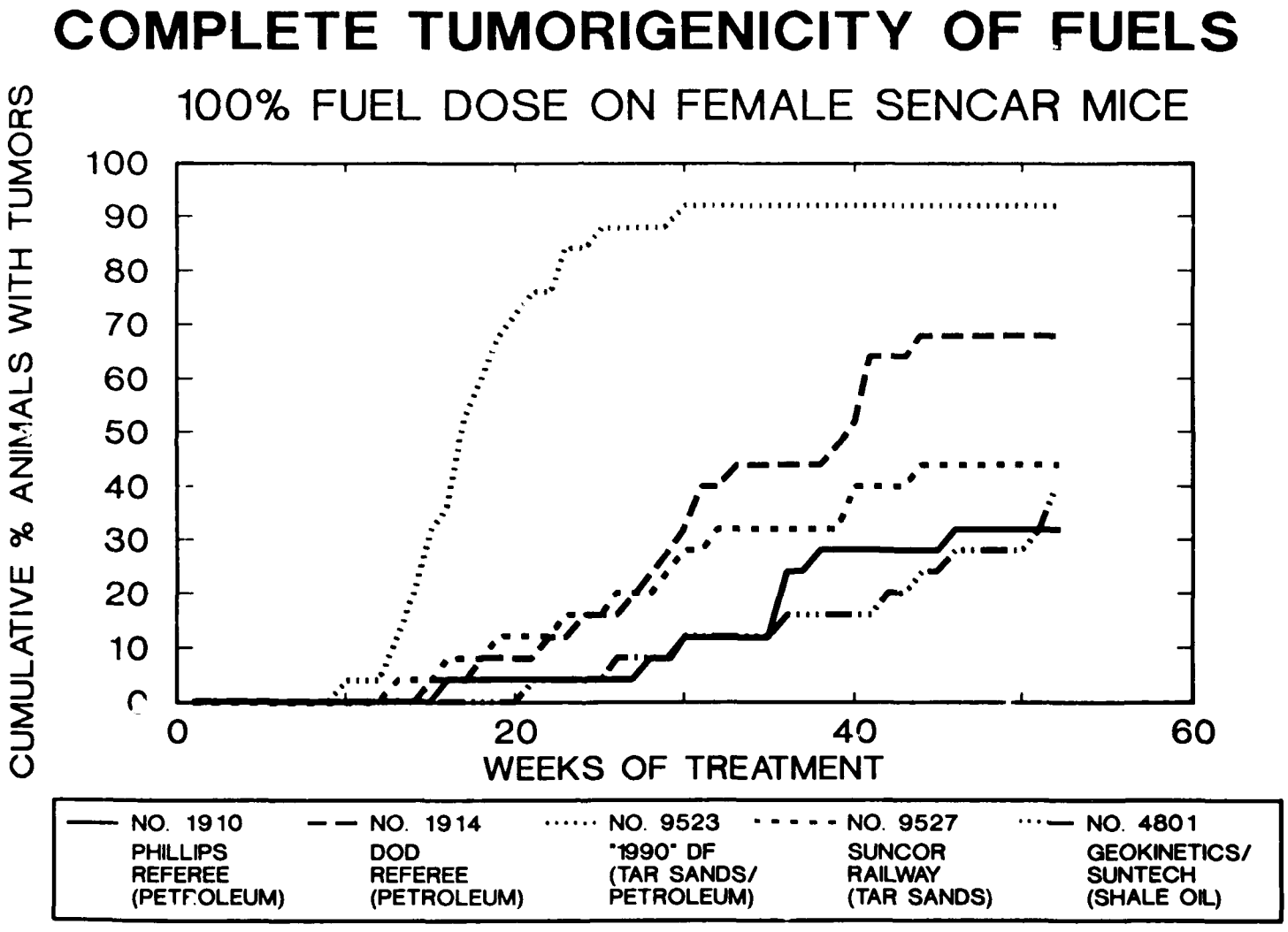

Figure 4. Cumulative Tumor Incidence (Female Sencar Mice) in the Comparative Complete Tumorigenicity Bioassay of Diesel Fuels Derived from Petroleum and Synthetic Sources

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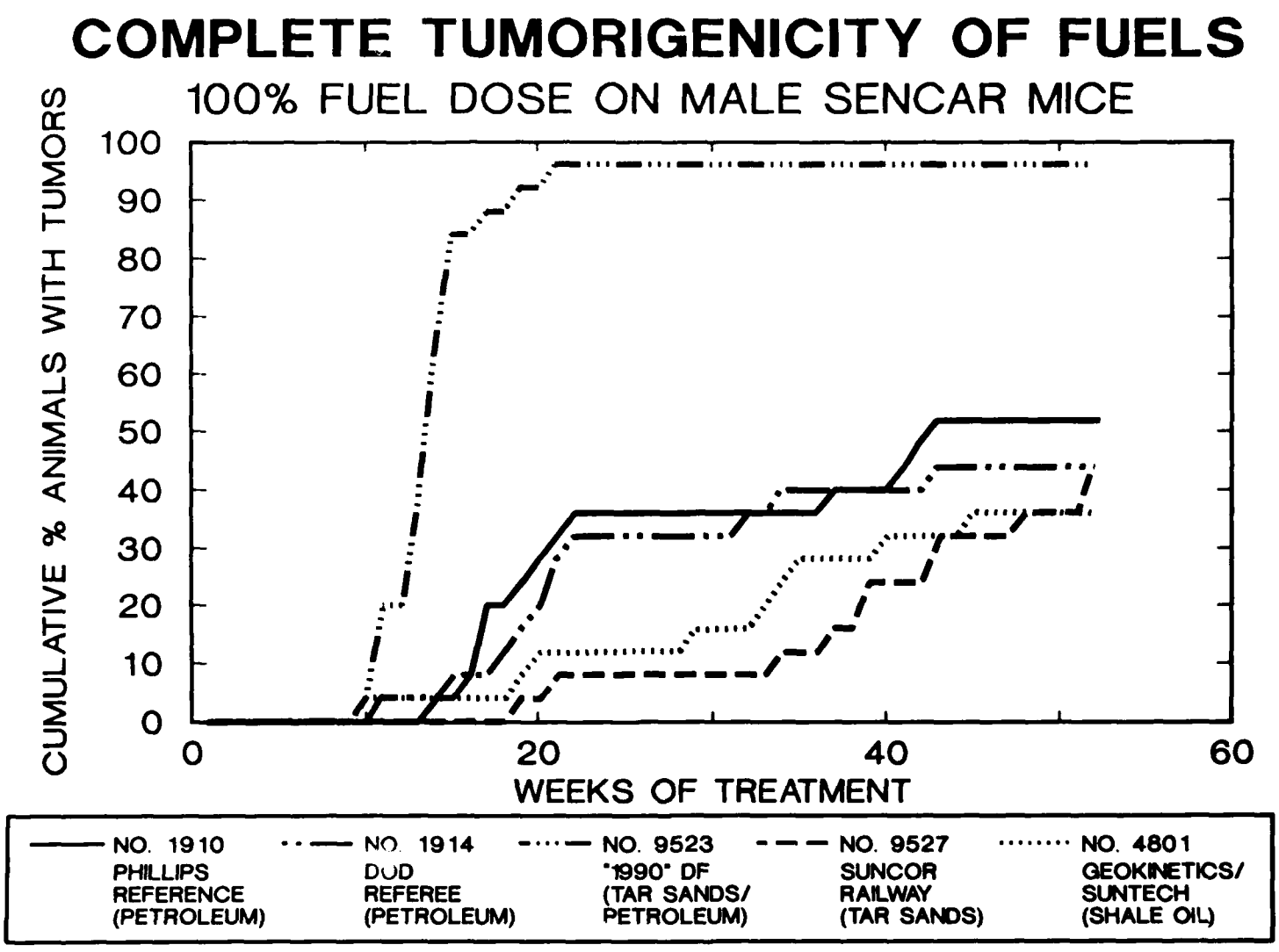

Figure 5. Cumulative Tumor Incidence (Male Sencar Mice) in the Comparative Complete Tumorigenicity Bioassay of Diesel Fuels Derived from Petroleum and Synthetic Sources

Table 2. Cumulative Tumor Incidence in the Comparative Tumor Promotion Bioassay of Petroleum- and Synthetically-Derived Diesel Fuels

Study Week	Phillips Reference DF-2 (Petroleum) (No. 1910)		DOD Referee Grade DF-2 (Petroleum) (No. 1914)		Canadian Research Council "1990 DF" (Tar Sands/Petroleum) (No. 9523)		Suncor Railway DF (Tar Sands) (No. 9527)		Geokinetics/Suntech DF-2 (Shale Oil) (No. 4801)		Positive Control DMBA/TPA		Negative Controls DMBA/Acetone		Negative Controls Acetone/TPA		Study Week
	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
6	0	0	0	4	0	8	0	4	0	4	4	4	0	0	0	0	6
8	8	36	12	44	8	32	8	32	0	4	64	68	0	0	0	0	8
10	28	72	40	60	20	52	16	36	8	24	84	92	0	12	0	0	10
12	40	80	56	80	60	88	28	40	16	44	92	92	0	16	4	0	12
14	52	84	76	84	72	100	52	48	24	48	100	100	0	24	8	0	14
16	68	84	76	92	76		68	48	24	56			0	32	8	0	16
18	68	84	80	96	88		76	56	24	64			0	36	8	0	18
20	72	84	88	96	88		80	60	24	64			0	40	8	0	20
22	76	84	88	96	88		80	60	24	68			0	44	8	0	22
24	76	84	88	96	88		84	60	28	68			0	44	8	0	24
26	76	84	88	96	88		84	60	28	68			0	44	8	0	26
28	92	84	88	96	88		88	64	36	88			0	44	20	8	28
30	92	88	88	96	88		88	64	44	88			0	44	20	8	30
32	92	92	88	96	88		88	68	44	92			0	44	28	8	32
34	92	92	88	96	88		92	72	52	92			4	60	28	12	34
36	92	92	88	96	88		92	72	52	92			4	60	32	16	36
38	92	96	88	96	88		92	72	52	92			4	64	32	16	38
40	92	96	88	96	88		92	72	60	92			4	64	44	20	40
42	92	96	88	96	88		92	72	64	92			4	64	48	20	42
44	92	96	88	96	88		92	72	68	92			4	64	52	24	44
46	92	96	88	96	88		92	72	68	92			8	64	52	24	46
48	92	96	88	96	88		92	72	68	92			8	64	52	24	48
50	92	96	88	96	88		92	72	68	92			8	64	52	28	50
52	92	96	92	96	92		92	72	80	92			8	68	60	28	52

the tumor promotion and complete tumorigenicity protocols. Detailed tables of the cumulative tumor incidence on a biweekly basis are contained in Tables 2 through 5.

Table 3

Comparison of Tumor Latency in the Tumor Promotion Bioassay of DF

Sample	Time to First Tumor, Wks		TT_{50},[a] Wks	
	M	F	M	F
No. 1910 Phillips Reference Df-2	7	8	9	14
No. 1914 DOD Referee DF-2	5	8	9	11
No. 9523 "1990" DF	6	7	10	12
No. 9527 Suncor Railway DF	6	8	13	14
No. 4801 Geokinetics/Suntech DF-2	6	10	14	34
DMBA + TPA	6	6	8	8
DMBA + Acetone	10	33	27	-
Acetone + TPA	24	11	-	44
No. 978 H-Coal Home Heating Oil	7	12	18	19
No. 975 API No. 2 Fuel Oil	8	8	12	16
No. 936 H-Coal Reformed Naphtha	5	10	52	-
No. 976 API Lt. Cat. Cracked Naphtha	11	11	19	-

[a] TT_{50} = time to 50% of the final tumor incidence; "-" means indeterminant.

Tumor Promotion Activity: The tumor promoting activity of the fuels is compared in Figures 1 and 2 and Tables 2 and 3. All the fuels exhibited tumor incidences greater than those of the negative controls (see Figure 3). The male animals tended to exhibit a greater tumor incidence than did the female animals for most of the DF dosing groups. By the end of the treatment period (52 weeks), tumor incidence for nearly all of the DF dosing groups was greater than 90 percent. These observations confirm the tumor promotion activity detected in a previous SENCAR mouse study (9) of an earlier production lot of Phillips (petroleum) Reference DF-2. That study also noted a greater response for the male animals. The cumulative tumor incidence for that study was 60 percent at 30 weeks versus the 90 percent determined here for a later production lot. It is not clear if the difference in activity is a result of the different lots of fuel, the different sources of the SENCAR mice, or both.

There was a sharp rise in tumor incidence for each of the fuels at ca. 8-10 weeks post initiation. The greatest differences in the cumulative tumor incidence were observed between ca. 15 and 25 weeks of treatment. During this period, the no. 9523 1990 DF (tar sands/petroleum) showed the greatest tumor promoting activity, closely followed by the no. 1914 DOD Referee DF (petroleum)for both the male and female mice. The no. 1910 Phillips Reference DF (petroleum) was intermediate in activity in both sexes. The no. 4801 Geokinetics/Suntech DF (shale oil) was the least active with the female mice while the no. 9527 Suncor Railway DF (tar sands) was least active with the male mice. By 52 weeks of treatment, tumor incidence was substantial and differences in activity were not as pronounced. This high tumor incidence reflects, in part, the high sensitivity of this strain.

The tumorigenic latencies (Table 3) were quite similar for all the DF when the time to first tumor is considered. This included the most active DF, the no. 9523 1990 DF derived from tar sands/petroleum coprocessing. However, the less active tar sands and shale oil-derived DF exhibited slightly longer latencies as expressed by the time to 50 percent of the final tumor incidence (TT_{50}). The previous study of an earlier production lot of Phillips Reference DF-2 remarked (9) on an unusual difference of 8 to 11 weeks in the tumor latency periods of the male and female animals, with the males exhibiting a time to first tumor of 10 weeks, and the females, 22 weeks. The latter is considerably greater than that observed in this study, and may be a result of the stronger tumorigenic response observed here. The results for the coal-and other petroleum-derived fuels will be discussed in the next subsection.

Complete Tumorigenicity: The complete tumorigenicity of the fuels is compared in Figures 4 and 5 and Tables 4 and 5. The main observation is that the no. 9523 1990 DF was considerably more tumorigenic than the other fuels in the tests with both sexes of mice. This greater activity appears to be related to its higher concentrations of

Table 4. Cumulative Tumor Incidence in the Comparative Complete Tumorigenicity Bioassay of Petroleum- and Synthetically-Derived Diesel Fuels

Study Week	Phillips Reference DF-2 (Petroleum) (No. 1910)		DOD Referee Grade DF-2 (Petroleum) (No. 1914)		Canadian Research Council "1990 DF" (Tar Sands/Petroleum) (No. 9523)		Suncor Railway DF (Tar Sands) (No. 9527)		Geokinetics/Suntech DF-2 (Shale Oil) (No. 4801)	
	F	M	F	M	F	M	F	M	F	M
2	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	4	4	0	0	0	4
12	0	0	0	4	4	20	0	0	0	4
14	0	4	0	4	16	60	4	0	0	4
16	4	8	4	8	32	80	12	0	0	4
18	4	20	8	12	60	88	12	0	0	4
20	4	28	8	20	72	88	16	4	0	12
22	4	32	8	28	76	92	16	8	4	12
24	4	32	12	28	80	92	20	8	4	12
26	4	32	12	28	84	92	20	8	4	12
28	8	36	24	36	88	96	20	8	4	12
30	12	36	32	36	92	96	28	8	12	16
32	12	36	40	36	92	96	32	8	12	16
34	12	36	44	40	92	96	32	12	12	24
36	24	36	44	40	92	96	32	12	16	28
38	28	40	44	40	92	96	32	16	16	28
40	28	40	48	40	92	96	40	24	16	32
42	28	48	64	40	92	96	40	24	20	32
44	28	52	68	44	92	96	44	32	24	32
46	32	52	68	44	92	96	44	32	28	36
48	32	52	68	44	92	96	44	36	28	36
50	32	52	68	44	92	96	44	36	28	36
52	32	52	68	44	92	96	44	44	40	36

tumorigenic PAH such as benzo[a]pyrene (BaP, see next section). The complete tumorigenicity of the remaining fuels was much lower, with the no. 1914 DOD Referee DF next in potency, with the female mice, while the no. 1914 and the no. 1910 Phillips Reference DF were next in potency for the male mice. The similarity between the potencies for the no. 1910 Phillips Reference DF-2 and the no. 9527 Suncor Railway DF are consistent with earlier findings (14) that distillate fractions of a tar sands crude oil were approximately as tumorigenic as the equivalent cuts from a petroleum crude oil. The no. 4801 Geokinetics/Suntech DF was least in potency with the female mice while the no. 9527 Suncar Railway DF was least potent with the male mice. The same order of potencies observed in the promotion and complete tumorigenicity assays suggests that tumor promotion may be important to the complete tumorigenicity of the fuels.

Table 5

Comparison of Tumor Latency in the Complete Tumorigenicity Bioassay of DF

Sample	Time to First Tumor, Wks		TT_{50},[a] Wks	
	M	F	M	F
No. 1910 Phillips Reference DF-2	14	15	43	-
No. 1914 DOD Referee DF-2	11	15	-	40
No. 9523 "1990" DF	10	10	14	17
No. 9527 Suncor Railway DF	19	13	-	-
No. 4801 Geokinetics/Suntech DF-2	10	21	-	-
No. 978 H-Coal Home Heating Oil	14	31	-	-
No. 975 API No. 2 Fuel Oil	4	15	-	-
No. 936 H-Coal Reformed Naphtha	16	-	-	-
No. 976 API Lt. Cat. Cracked Naphtha	14	34	-	-

[a] TT_{50} = time to 50% of final tumor incidence; "-" means indeterminant.

The tumor latencies are compared in Table 5. As with the tumor promotion testing, no large differences were observed in the time to first tumor.

The previous study (9) applied Phillips DF once per week to the SENCAR mice for 38 weeks. No tumors were observed, suggesting that the less frequent application resulted in a dose below a tumorigenic threshold, and that a longer application period or more frequent dosing would be required to detect tumors. This observation illustrates the difficulties in bioassay of highly refined fuels which do not possess strong biological activities.

Comparison with Coal- and Other Petroleum-Derived Fuels

Data for tumor promotion testing of an additional petroleum-derived no. 2 fuel oil and naphtha product and two coal-derived analogs are shown in Figures 4 and 5. The tumor promoting activity of the no. 975 API no. 2 Fuel Oil (petroleum) was similar to that of the no. 1910 Phillips Reference DF, and is consistent with their very similar compositions (see next section). The no. 975 API no. 2 Fuel Oil gave the highest tumor incidence with the male mice, while the no. 978 H-Coal Home Heating Oil (coal liquid) showed the highest tumor promoting activity in the female mice. Both of the naphthas exhibited tumor promoting activities which were much lower than those of the DF/fuel oils and their responses were not appreciably different from those of the negative controls. Tumor latencies (see Table 3) were not different from those of the DF.

The results for the complete tumorigenicity testing of coal- and additional petroleum-derived fuels (Figures 8 and 9) show that the complete tumorigenicity of the no. 975 API no. 2 Fuel Oil is consistent with that of the petroleum-derived DF. The activity of the no. 978 H-Coal Home Heating Oil was lower than that of the petroleum derived no. 2 fuel oil and only slightly above that of the naphthas. In a lifetime study (5) using the C3H strain, the H-Coal Home Heating Oil was the most tumorigenic of these four fuels. Differences in the responses of samples between different strains or species of animals is common. Again, the tumor latencies of these additional fuels (see Table 5) are not particularly different from those of the DF.

The results of these dermal assays suggest that synthetically-derived DF-range fuels probably will not exhibit skin tumorigenicity greater than that of currently available petroleum-derived DF. Rather, the differences in toxicity are likely to be subtle. This includes fuels derived from shale oil, coal liquids, and tar sands. A possible exception is the technology for the tar sands/petroleum coprocessing-derived DF. The elevated toxicity of this product appears to be attributable to the petroleum-derived light cycle oil used in blending. It remains to be demonstrated experimentally that this is indeed the case, and that decreasing the blend of light cycle oil decreases the tumorigenicity of that fuel.

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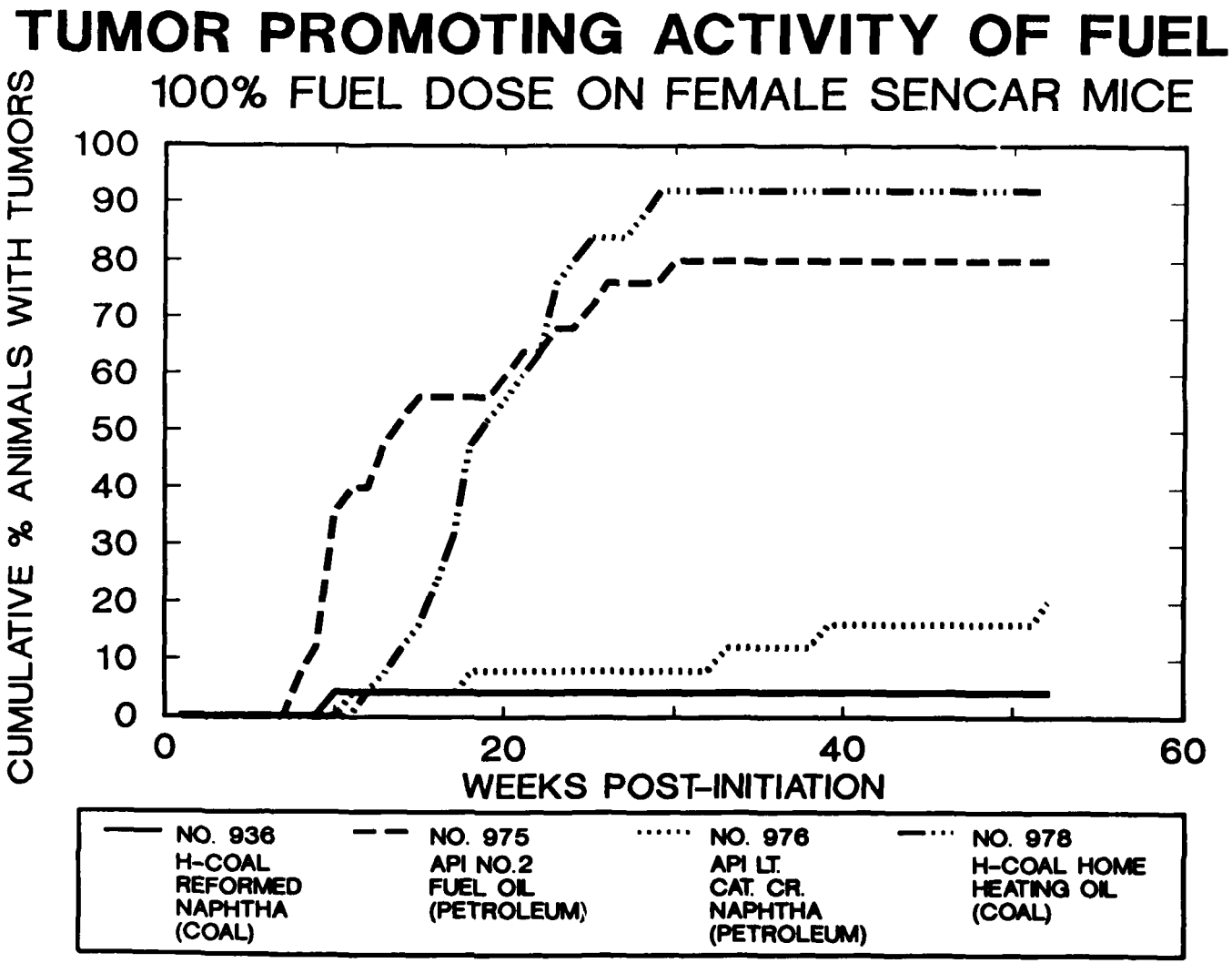

Figure 6. Cumulative Tumor Incidence (Female Sencar Mice) in the Comparative Tumor Promotion Bioassay of Additional Petroleum- and Coal-Derived Fuels for the DOE/FE

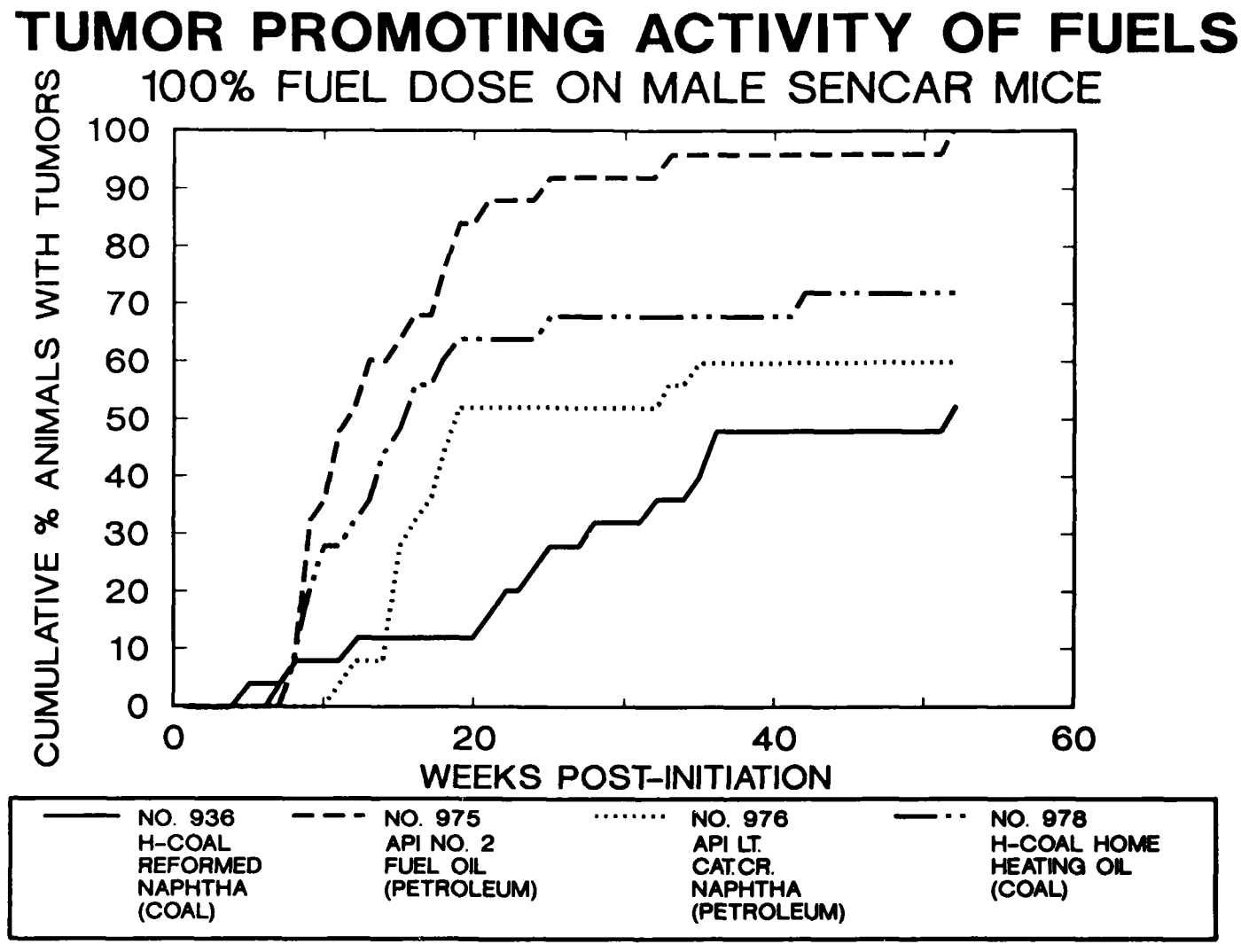

Figure 7. Cumulative Tumor Incidence (Male Sencar Mice) in the Comparative Tumor Promotion Bioassay of Additional Petroleum- and Coal-Derived Fuels for the DOE/FE

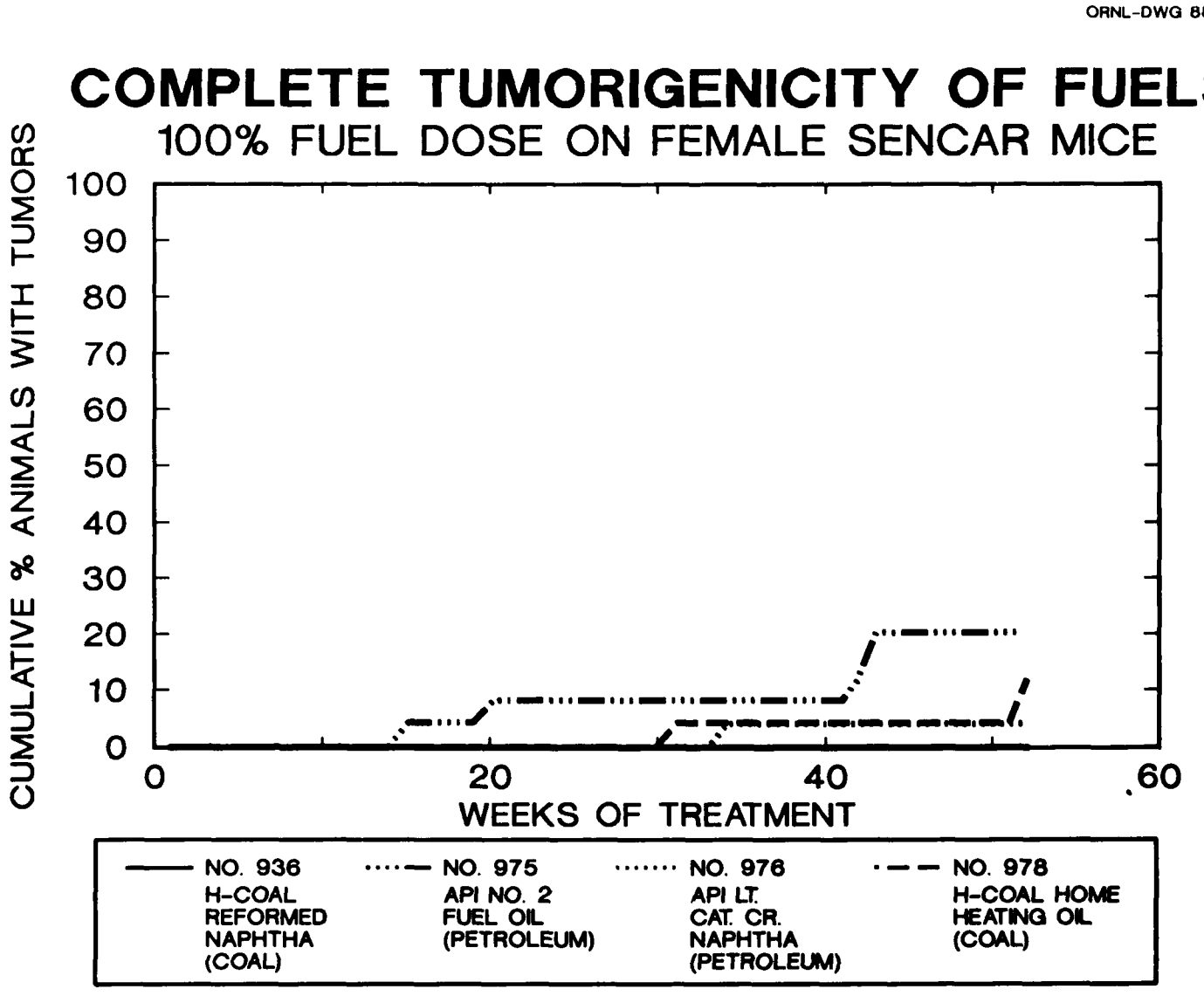

Figure 8. Cumulative Tumor Incidence (Female Sencar Mice) in the Comparative Complete Tumorigenicity Bioassay of Additional Petroleum- and Coal-Derived Fuels for the DOE/FE

ORNL-DWG 88Z-10812

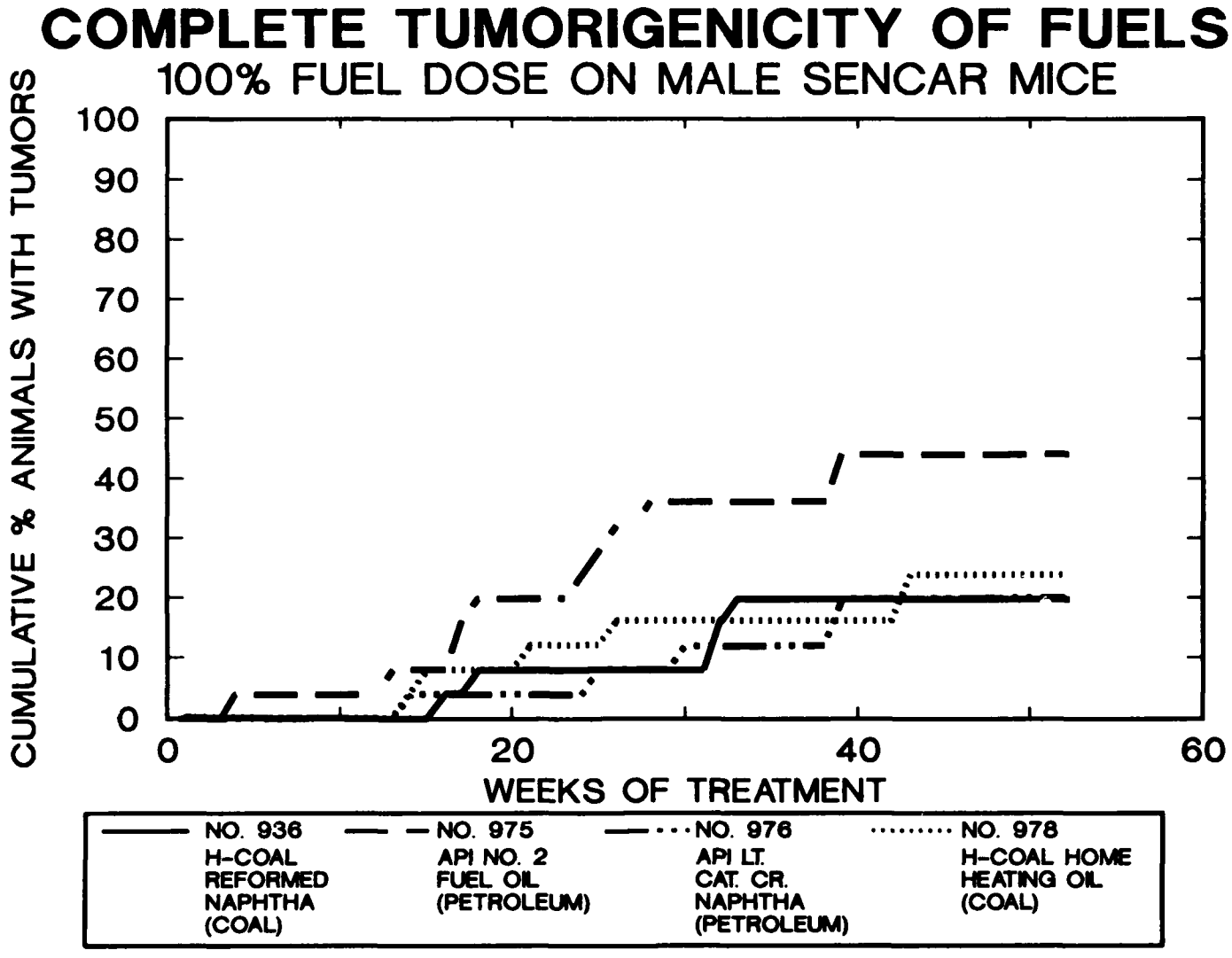

Figure 9. Cumulative Tumor Incidence (Male Sencar Mice) in the Comparative Complete Tumorigenicity Bioassay of Additional Petroleum- and Coal-Derived Fuels for the DOE/FE

CHEMICAL COMPARISON OF FUELS

A chemical comparison of the fuels and also of their inhalable vapors was conducted to determine if compositional differences existed between fuels derived from petroleum and synthetic sources. These data provide a better definition of the fuels and assist in the interpretation of the results of the skin painting assays. The data on the inhalable vapors also might indicate if major differences in inhalation toxicity would be expected.

Comparison of Major Organic Compound Composition of Fuel Liquids

The major organic compounds in the fuels were determined to define the bulk composition of the fuel liquids. Although most of the major organic compounds are not particularly toxic, the nature of the bulk liquid could affect the skin absorption and metabolism of more toxic fuel components. The analysis was by direct, high resolution capillary column gas chromatography (GC) of a diluted sample of the fuel, as described in detail elsewhere (15). An HP-5880 GC was equipped with a 60 m x 0.25 mm ID fused silica column coated with a 0.25 μm bonded film of DB-5, a flame ionization detector, splitless injector, and the HP Level IV data system (programmable in Basic). A 10 μL volume of fuel and 202 μg of 1,1'-binaphthyl internal standard (in 100 μL of methylene chloride) were diluted to 10 mL with methylene chloride, and 1 μL was injected in the splitless mode into the GC. The column oven was temperature programmed from 50°C (initial 10 min. isothermal hold) to 250°C at 2°C/min. and held at 250°C for 20 min. with a hydrogen carrier gas flow rate of 1.4 mL/ min. The injector and detector temperatures were 200°C and 250°C, respectively. Quantitation of known in the fuels was achieved using the method of internal standards. Selected fuels were examined under similar chromatographic conditions by gas chromatography-mass spectroscopy (GC-MS) to confirm the tentative identifications made by GC.

Figure 10 is a chromatogram of the no. 1910 Phillips (petroleum) Reference DF-2. The GC-MS identification of the peaks is listed in Table 6 along with the estimated concentrations. This is a typical petroleum-derived DF-2. The major organic compounds consist of a series of n-paraffins ranging from ca. C_8 through at least C_{25}. The 2-methyl naphthalene, 1-methyl naphthalene, several dimethyl naphthalenes (including the 1,3-, 1,5-, and 1,4-isomers), pristane, and phytane also are among the major constituents. Other branched hydrocarbons and numerous alkylated benzenes, indanes, naphthalenes, tetrahydronaphthalenes, biphenyls/acenaphthalenes, and phenanthrenes comprise the remainder of the identified constituents which accounted for ca. 46 percent of the fuel mass. Detailed fractionation studies (8, 16-20) have established the identification of such compounds in petroleum-derived DF. The minor constituents are of considerable importance because the major compositional diffferences among the fuels were in the concentrations of these constituents.

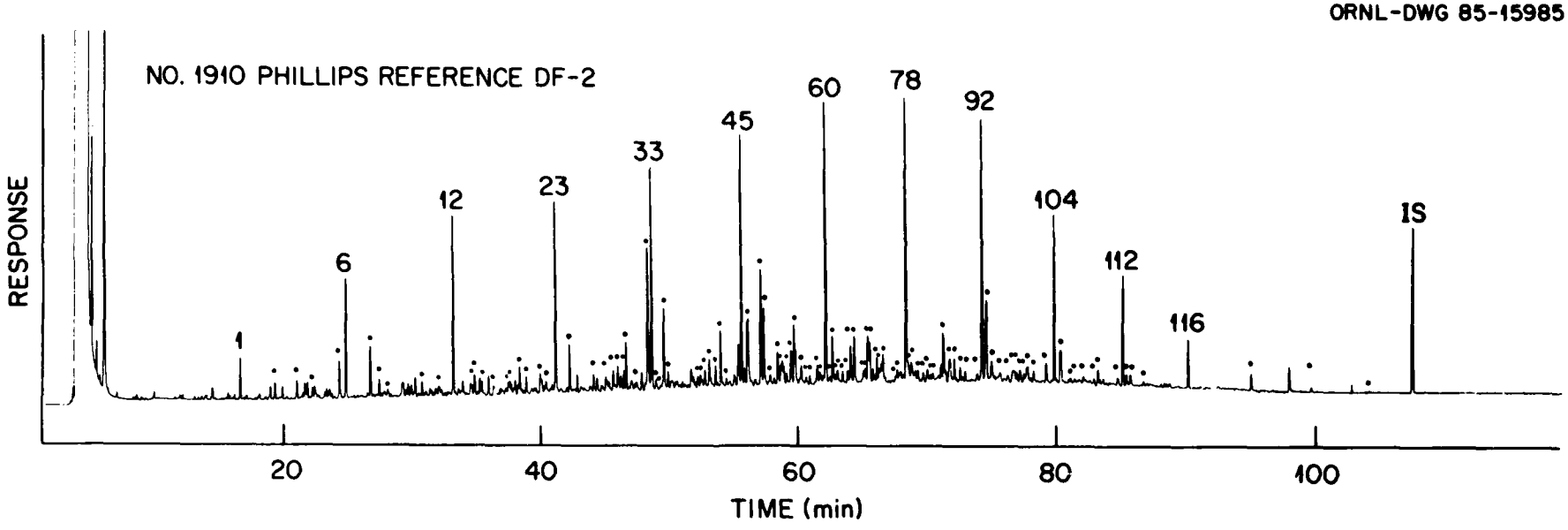

Figure 10. Capillary Column Gas Chromatographic Separation of the Major Organic Compounds in No. 1910 Phillips Reference DF-2. (Component identifications in Table 6. GC column: 60 m DB-5, temperature programmed from 50°[10 min. isothermal hold] to 250° at 2°/min. and held at 250°C for 20 min.)

Table 6

Identification and Estimation of the Major Organic Compounds in No. 1910 Phillips Reference DF-2

Peak No.[a]	Tentative Identification[b]	Concentration[c], mg/g
1	n-C_9 H_{20}	5.0
2	Hydrocarbon	1.7
3	Hydrocarbon	1.8
4	C_3-Benzene	1.3
5	C_3-Benzene + Hydrocarbon	3.5
6	n-C_{10} H_{22}	10.6
7	Hydrocarbon, possibly branched C_{11}	5.0
8	C_3-Benzene	1.7
9	C_4-Cyclohexane	1.1
10	Hydrocarbon + C_4-Benzene	1.5
11	C_4-Benzene	0.8
12	n-C_{11} H_{24}	16.7
13	C_4-Benzene	1.1
14	C_4-Benzene + Hydrocarbon	1.9
15	Hydrocarbon	1.7
16	Hydrocarbon	1.9
17	C_1-Indane	1.3
18	C_4-Benzene	1.4
19	Hydrocarbon	2.8
20	Hydrocarbon, possibly 3-Methyl-C_{11}	1.7
21	Naphthalane	0.9
22	C_2-Indane	0.8
23	n-C_{12} H_{26}	18.5
24	Hydrocarbon	4.9
25	Hydrocarbon	1.7
26	C_2-Indane + Hydrocarbon	1.5
27	Hydrocarbon	2.1
28	Hydrocarbon, maybe 2-Methyl-C_{12}	2.5
29	C_2-Indane + Hydrocarbon	1.9
30	Hydrocarbon	4.8
31	C_5-Benzene + Unknown	0.5
32	2-Methyl Naphthalene	14.9
33	n-C_{13} H_{28}	22.5
34	C_3-Indane	1.1
35	Hydrocarbon	<0.5
36	1-Methyl Naphthalene	8.1
37	C_2-Tetrahydronaphthalene	1.5

[a] Figure 6

[b] Specific isomer listed when retention time and mass spectrum agree with authentic standards. Generic identifications are tentative and other isomeric assignments are possible.

[c] Concentration estimates for generically identified species should be considered semiquantitative (±20% or more).

Table 6

Identification and Estimation of the Major Organic Compounds in No. 1910 Phillips Reference DF-2

Peak No.[a]	Tentative Identification[b]	Concentration[c], mg/g
38	Hydrocarbon	1.3
39	Hydrocarbon	1.3
40	Hydrocarbon	1.7
41	Hydrocarbon	2.6
42	Hydrocarbon, maybe 3-Methyl-C_{13}	1.9
43	Hydrocarbon	5.9
44	Biphenyl	0.7
45	n-C_{14} H_{30}	24.6
46	C_2-Naphthalene	6.7
47	1,3-Dimethyl Naphthalene	12.8
48	C_2-Naphthalene	7.6
49	Hydrocarbon	1
50	1,5-Dimethyl Naphthalene	3.7
51	1,4-Dimethyl Naphthalene	2.1
52	Hydrocarbon	1
53	Hydrocarbon	3.1
54	Hydrocarbon, maybe 2-Methyl-C_{14}	5.5
55	Hydrocarbon	1.7
56	Hydrocarbon	0.7
57	Hydrocarbon	1.1
58	C_3-Naphthalene	1.6
59	C_1-Biphenyl	0.8
60	n-C_{15} H_{32}	30.9
61	C_3-Naphthalene	0.4
62	C_3-Naphthalene	4.5
63	C_3-Naphthalene	0.4
64	C_3-Naphthalene	1.1
65	C_3-Naphthalene	1.1
66	C_3-Naphthalene	1.3
67	C_3-Naphthalene	3.6
68	C_3-Naphthalene	4.6
69	Hydrocarbon	1.2
70	Hydrocarbon	1.4
71	C_3-Naphthalene	4.9
72	C_3-Naphthalene	4.1
73	C_3-Naphthalene	2.5
74	C_3-Naphthalene	1
75	Hydrocarbon, maybe 3-Methyl C_{15}	2.5
76	C_3-Naphthalene	1
77	Fluorene	1.3
78	n-C_{16} H_{34}	28.8
79	C_1-Biphenyl/C_1-Acenaphthene + C_4-Naphthalene	2
80	C_2-Biphenyl/C_2-Acenaphthene	1.9
81	C_1-Biphenyl/C_1-Acenaphthene + C_4-Naphthalene	1.0
82	C_4-Naphthalene	0.9

Table 6

Identification and Estimation of the Major Organic Compounds in No. 1910 Phillips Reference DF-2

Peak No.[a]	Tentative Identification[b]	Concentration[c], mg/g
83	C_4-Naphthalene	1.1
84	C_4-Naphthalene	0.6
85	C_2-Biphenyl/C_2-Acenaphthene	0.7
86	Hydrocarbon	4.9
87	Hydrocarbon + C_4-Naphthalene	2.3
88	Hydrocarbon	2.3
89	Hydrocarbon	1.5
90	C_4-Naphthalene	1.4
91	C_4-Naphthalene	0.9
92	n-C_{17} H_{36}	25.0
93	Pristane	8.0
94	1-Methyl Fluorene + C_2-Biphenyl/C_2-Acenaphthene	2.1
95	C_1-Fluorene + C_2-Biphenyl/C_2-Acenaphthene	0.7
96	C_2-Biphenyl/C_2-Acenaphthene	0.8
97	Hydrocarbon + C_2-Biphenyl/C_2-Acenaphthene	1.2
98	Hydrocarbon	0.8
99	Hydrocarbon	1.2
100	Hydrocarbon	0.8
101	C_4-Naphthalene + Hydrocarbon	1.6
102	Hydrocarbon	1.4
103	Phenanthrene	2.3
104	n-C_{18} H_{38}	20.0
105	Phytane	5.8
106	C_3-Biphenyl/C_3-Acenaphthene	0.5
107	C_3-Biphenyl/C_3-Acenaphthene	0.5
108	Hydrocarbon + C_4-Biphenyl/C_4-Acenaphthene	0.5
109	Hydrocarbon	0.5
110	Hydrocarbon + C_4-Biphenyl/C_4-Acenaphthene	1.3
111	Hydrocarbon	0.6
112	n-C_{19} H_{40}	11.9
113	C_1-Phenanthrene	1.3
114	2-Methyl Phenanthrene	1.2
115	C_1-Phenanthrene	0.5
116	n-C_{20} H_{42}	5.4
117	n-C_{21} H_{44}	2.2
118	n-C_{22} H_{46}	0.7
119	n-C_{23} H_{48}	0.4
IS	Internal Standard (1,1'-Binaphthyl)	-
	TOTAL	459.9

Figure 11 is a comparison of the chromatograms for the major organic compounds in the three major DF types examined in this study: the no. 1910 Phillips (petroleum) Reference DF-2 (top), the no. 4801 Geokinetics/Suntech (shale oil) DF-2 (middle), and the no. 9523 Canadian 1990 (tar sands/petroleum) DF (bottom). It is evident that the three fuels were similar in qualitative composition, but quite different quantitatively. The shale oil-derived fuel was characterized by a very low content of diaromatics while the tar sands/petroleum coprocessing DF was relatively high in diaromatics. This is readily visualized by comparing the monomethyl naphthalenes and n-$C_{13}H_{28}$ (n-C_{13}). The peak for n-C_{13} is indicated with a dot in Figure 11. The peaks immediately to the left and right are for 2- and 1-methyl naphthalene, respectively.

The compositional differences suggested in Figure 11 appear to be generic at least for the petroleum and shale oil-derived DF. Chromatograms for five other petroleum-derived DF-2 and two other shale oil-derived DF included in the Appendix show this same generic difference. Not enough examples of tar sands-derived fuels were available to determine their common compositional characteristics. However, conversations with staff of the Canadian National Research Council indicated that the high concentrations of the polycyclic aromatics in the 1990 DF were contributed mainly by the petroleum-derived light cycle oil used in blending, and not the tar sands component. It is probable that reduction of the blending volume of the former or use of a hydrotreated petroleum stream would significantly decrease the aromatics (especially PAH) content.

The major organic compounds quantitatively determined in the DF are listed in Table 7. The data for these five DF plus those for seven additional DF obtained from the Phillips Chemical Co., Fort Carson, WPAFB, and SOHIO confirm these generic compositional differences noted above in the comparison of the gas chromatograms. The precision of the quantitative determinations was estimated to be ca. $\pm$ 2 to 7 percent. Whereas the petroleum- and shale oil-derived DF exhibited very similar concentrations of the n-paraffins and alkyl benzenes (see below), the concentrations of the higher alkylated ($> \approx C_3$) benzenes, the mono- and dimethyl naphthalenes, and the phenanthrenes was much higher in the petroleum-derived DF. In contrast, the 1990 DF derived from tar sands/petroleum coprocessing was distinctly different from either the petroleum- or shale oil-derived DF. It was characterized by a relatively high ratio of aromatics to aliphatics, and a low ratio of pristane and phytane to n-C_{17} and n-C_{18}, respectively. The n-paraffins in the midrange (ie., C_{10} - C_{19}) are ca. 30-50 percent as concentrated as those in the petroleum- and shale oil-derived DF, while the alkyl naphthalene concentrations are very similar to those of the petroleum fuels. However, n-paraffins above C_{17} and below C_{10} are more concentrated than in the petroleum or shale oil fuels. The tar sands-derived railway DF also exhibits an overall lower concentration of paraffins, but it lacks the aromatics content of the petroleum- and tar sands/petroleum coprocessing-derived DF.

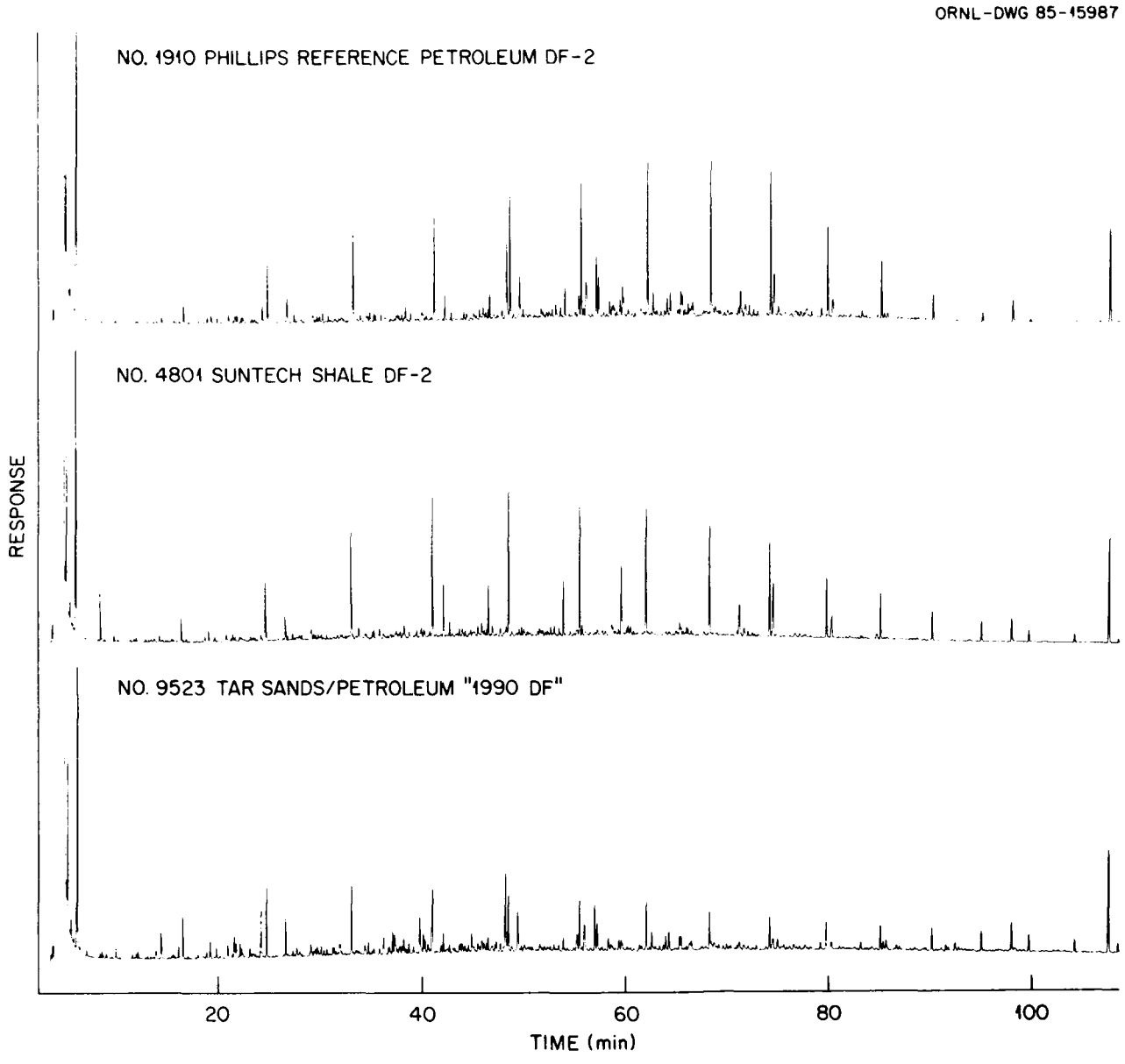

Figure 11. Comparison of the Major Organic Compounds in Diesel Fuels Derived from Petroleum, Shale Oil, and Tar Sands/Petroleum Coprocessing

Table 7

Comparison of the Major Organic Compounds (in mg/g) in Diesel Fuels Derived from Petroleum, Shale Oil, Tar Sands, and Tar Sands-Petroleum Coprocessing

	Petroleum-Derived DF-2 or DFM							Shale-Derived DF2 or DFM			Petroleum Tar Sands Co-Proces.	Tar Sands
Sample	1910	9101	1914	DF-2-1	DF-2-2	DF-2-3	4616	4801	4802	4610	9523	9527
Compound	Phillips Lot C745	Phillips Lot C345	DOD Referee	Ft. Carson DIO	Ft. Carson AMP	Ft. Carson EMP	WPAFB DFM	Geokinetics Suntech w/o Add.	Geokinetics Suntech w/ Add.	Paraho/ SOHIO DFM	Petrol. Tar Sands 1990 DF	Suncor Rail. DF
C_8	-	-	-	-	1.0	1.3	-	5.8	6.5	0.3	-	-
C_9	4.9	3.6	2.1	4.8	4.3	4.0	3.6	5.1	5.4	2.4	9.0	2.8
C_{10}	10.5	10.1	2.8	12.2	7.7	5.9	5.7	9.3	9.6	4.6	9.9	3.3
C_{11}	16.9	17.1	5.7	22.6	13.4	10.4	11.2	17.3	17.7	9.6	10.1	4.3
3Me-C_{11}	1.7	1.8	0.9	1.8	1.4	-	2.8	1.2	1.1	-	1.6	1.4
Naphthalene	1.3	1.6	2.5	2.0	1.9	1.2	2.2	1.7	1.8	0.9	5.7	<2.6
C_{12}	18.5	17.7	10.3	20.5	13.9	11.1	24.5	22.3	22.7	15.6	9.6	6.4
2Me-C_{12}	2.8	2.7	2.5	2.7	2.2	1.5	5.2	2.5	2.5	2.0	2.1	0.7
2Me-Nap	14.9	8.4	13.5	6.4	9.6	7.1	10.9	1.9	1.9	1.9	12.7	<1.8
C_{13}	22.6	20.4	20.2	21.7	16.7	14.8	28.2	24.2	24.8	25.4	8.1	3.5
1Me Nap	8.1	4.6	8.1	3.4	4.7	3.8	5.9	1.1	1.3	1.6	5.8	<0.4
3Me-C_{13}	2.0	2.0	2.2	1.5	1.5	1.3	3.0	1.3	1.5	2.2	0.9	1.4
Biphenyl	-	-	1.2	-	-	-	-	-	-	-	-	-
C_{14}	24.8	20.8	25.4	19.3	19.1	18.8	27.0	21.4	21.5	28.8	7.6	3.9
1,3-DiMe Nap	12.8	8.6	12.3	5.5	9.4	8.5	10.5	1.0	1.0	1.3	8.0	2.4
1,5-DiMe Nap	3.6	2.7	3.6	1.6	2.8	2.6	3.1	-	-	-	2.4	-
1,4-DiMe Nap	2.2	1.8	2.3	1.1	1.6	1.6	2.1	1.8	1.8	2.7	1.2	-
2-Me C_{14}	5.5	5.0	5.8	3.4	3.8	3.9	6.3	11.3	11.4	14.9	0.9	1.1
C_{15}	30.9	26.2	25.2	19.0	24.0	26.3	29.4	20.6	20.6	28.9	7.0	5.1
Fluorene	1.3	1.4	1.2	0.6	0.9	1.3	1.5	0.5	0.4	1.1	0.7	-
C_{16}	28.5	24.8	19.6	14.9	21.9	25.7	26.2	19.2	19.2	27.8	6.0	3.0
C_{17}	25.1	23.6	28.6	14.4	19.7	24.7	20.4	15.8	15.9	25.5	10.5	2.7
Pristane	8.1	7.4	6.0	3.5	4.7	5.8	6.7	9.7	9.8	17.1	1.9	0.5
Phenanthrene	2.4	3.0	1.9	-	1.9	1.7	1.8	-	-	-	1.6	-
C_{18}	19.7	17.0	12.3	11.8	16.0	19.3	14.6	12.2	12.4	21.5	5.2	2.1
Phytane	5.9	5.5	5.3	3.5	4.9	5.7	4.1	7.1	7.1	13.8	2.3	0.4
C_{19}	11.9	9.2	7.3	9.2	11.7	14.7	8.2	8.8	8.7	9.0	4.5	1.4
C_{20}	5.4	3.7	4.0	6.4	8.4	10.1	5.1	5.8	5.7	-	4.3	0.7
C_{21}	2.3	1.6	2.4	5.5	7.0	8.3	3.7	5.1	5.1	-	4.7	0.4
2Me Phen	1.4	1.6	1.7	-	1.8	1.6	1.6	-	-	-	2.1	-
C_{22}	-	-	-	2.9	3.8	4.4	1.4	2.5	2.4	-	3.4	-
C_{23}	-	-	-	1.9	2.4	2.8	-	2.0	1.8	-	2.8	-
C_{24}	-	-	-	-	-	-	-	-	-	-	1.9	-
C_{25}	-	-	-	-	-	-	-	-	-	-	2.0	-
TOTAL ID	296	255	237	224	245	249	277	239	241	253	156	52.3

The benzene and alkyl benzene content of the fuels was compared also because of their known toxicity (21). For this measurement, a 200 μL aliquot of DF and 1.62 μg of tetrachloroethylene internal standard were diluted to 10 mL with diethyl ether. The same GC as for the major organic compounds was used for the benzene and alkyl benzenes measurements, but the temperature program was changed to 20°C (15 min. isothermal hold) to 75°C at 1°C/min. and then to 250°C at 20°C/min. The injector and detector were maintained at 150°C and 250°C, respectively. The procedure is described in more detail in reference (15). The identifications were confirmed by GC-MS under similar chromatographic conditions. Data for five of the fuels are presented in Table 8. With the exception of the toluene in the Geokinetics/-Suntech DF-2, the concentrations of these compounds in the petroleum- and shale oil-derived DF were quite similar. This observation suggests that similar concentrations of these compounds (except for toluene) would be found in the inhalable volatiles from these fuels. The data for the no. 975 API No. 2 Fuel Oil were consistent with those for the petroleum DF, as expected from their common petroleum sources and similar boiling ranges. However, the coal-derived no. 978 H-Coal Home Heating Oil contained much higher concentrations of benzene and alkyl derivatives, reflecting the more aromatic nature of the coal liquids versus crude petroleum. This suggests that the inhalable volatiles from the coal liquids-derived product may contain greater concentrations of aromatics.

A comparison of selected 4- to 6-ring PAH dermal tumorigens was conducted to provide data on these potent tumor initiators and complete carcinogens which would aid in interpretation of the skin painting bioasssay results. The known (22) contribution of fuel PAH to diesel engine exhaust PAH was another important reason for this comparison. Two analytical procedures were used. A sequential high performance liquid chromatography (HPLC) procedure (23) consisting of a semipreparative scale, normal phase HPLC fractionation followed by an analytical scale reverse phase HPLC with fluorescence detection was applied to the determination of benzo[a]pyrene (BaP) in all the fuels. The fuel, spiked with carbon-14 labeled BaP, was fractionated on a 25 cm x 10 mm ID Partisil PAC-10 column using an eluent (2 mL/min.) of methylene chloride/hexane (1/9, vol./vol. for 30 min.) followed by column washes with neat methylene chloride (30 min.), acetonitrile/methylene chloride (66/33, vol./vol., for 30 min.), methylene chloride (30 min.) and methylene chloride/hexane (1/9, vol./vol., for 30 min.). The BaP-enriched fraction was analyzed on an 8 cm x 6.4 mm ID Golden Series octadecylsilane column using an acetonitrile/water (75/75, vol./vol. at 2.2 mL/min.) mobile phase and fluorescence detection with 360 nm excitation and 425 nm emission wavelengths. Quantitation was by the method of external standards. The recovery of BaP was determined by liquid scintillation counting the added carbon-14 labeled BaP. A separate procedure (24) involving semipreparative scale, normal phase HPLC followed by GC-MS with selected ion monitoring was used for a more comprehensive analysis of

TABLE 8

COMPARISON OF THE BENZENE/ALKYL BENZENE CONTENT OF
DIESEL FUELS AND FUEL OILS FROM NATURAL AND SYNTHETIC SOURCES

	Concentration in Fuel, mg/g[a]						
	Petroleum				Shale		Coal
Compound	1910 Phillips	1914 DOD Reference	DF-2-1 DIO	975 API No.2 Fuel Oil	4801 Geokinetics Suntech	4610 Paraho SOHIO DHM	978 Home Ht. Oil
Benzene	0.026	0.082	0.048	<0.02	0.01	0.027	2.9
Toluene	0.27	0.83	0.69	0.3	4.7	0.25	3.3
Ethyl Benzene	0.17	0.43	0.39	0.2	0.26	0.20	2.6
m+p-Xylen	1.3	2.0	2.5	2.1	1.0	0.66	3.5
Styrene	<0.04	<0.02	<0.05	-	<0.06	<0.02	-
o-Xylene	0.42	0.78	0.85	0.6	0.32	0.24	1.5
i-Propyl Benzene	<0.1	<0.2	IR	<0.3	-	IR	1.3
n-Propyl Benzene	0.30	0.40	0.48	0.2	0.15	0.12	2.4
1,3,5-Trimethyl Benzene	2.0	0.90	2.4	<2	0.87	0.43	<0.5
4-i-Propyl Toluene	0.26	0.03	IR	-	IR	IR	-
n-Butyl Benzene	0.31	0.46	IR	<0.7	IR	IR	<1

[a]IR = incomplete resolution prevented measurement

certain 4- to 6-ring PAH in the five main fuels. The isolation of the PAH-enriched fraction was similar to that described above for BaP, except that a 25 cm x 9.4 mm ID cyano-substituted silane stationary phase and hexane (16 min.) and methylene chloride/hexane (15/25 vol./vol., for 36 min.), and methylene chloride/hexane (40/60 vol./vol., 20 min.), followed by pure methylene chloride (30 min.) mobile phases were used at 2.25 mL/min. in the normal phase HPLC. The fraction eluting between 26 min. and 66 min. was collected. GC-MS employed a 30 m x 0.25 mm ID x 0.25 μm film of DB-5, temperature programmed from 150° (3 min. isothermal hold) to 290°C at 2°C/min. with a helium carrier gas flow rate of ca. 1 mL/min. Quantitation was by the method of internal standards using perdeuterochrysene and perdeutero BaP which were added to the fuels prior to fractionation.

Results for the PAH determinations are presented in Tables 9 and 10. Considering the sub-μg/g concentrations of BaP in the fuels, the agreement between the two methods is quite reasonable. Considerable variation was observed in the BaP concentrations among the petroleum fuels. The DOD Referee DF-2 and the DF-2-1 petroleum DF-2 collected from the Fort Carson DIO were high, with BaP in the latter approaching 1 μg/g. The BaP content of petroleum-derived DF has been reported (25,26) to range from $<$ 0.001 to 0.42 μg/g. The petroleum-, tar sands-, and shale oil-derived fuels were lower than the tar sands/petroleum coprocessing 1990 DF and the H-Coal Home Heating Oil and Reformed Naphtha in BaP content. In particular, the 4.2 μg/g of BaP for the 1990 DF was very high for DF. As noted above, this PAH content appears to be contributed by the petroleum light cycle oil used in blending.

The data for the 4- to 6-ring PAH show that the DF high in BaP also are high in other tumorigenic PAH. The DOD Referee DF-2 contained somewhat higher levels of these PAH than did the Phillips Reference DF-2, which was more like the Geokinetics/Suntech and Suncor DF in PAH content. The 1990 DF was the most enriched in these PAH. The latter would be expected to exhibit greater tumorigenicity on this basis. It also would be expected (22) to contribute to higher levels of PAH in diesel engine exhaust, and on that basis, the exhaust could exhibit a greater inhalation hazard.

Comparison of Fuel Composition and Tumorigenicity

A comparison of selected bulk fuel liquid compositional data with the dermal tumorigenicity data is shown in Table 11. The comparison includes the ratio of the aromatics to saturates from the GC determination of major organics (from Table 7), the ratio of 2-methyl naphthalene to n-C_{13} (also from Table 7), the total volume percent of aromatics as determined by the fluorescent indicator assay (Table 11), the BaP (Table 9), and the sum of the 5- and 6-ring PAH dermal tumorigens (Table 10) versus the cumulative tumor incidence at 26 and 52 weeks in the tumor promotion and complete tumorigenicity protocols

Table 9

HPLC Determination of BaP Content of Fuels

Sample No.	Description	Concentration, μg/g
	---Shale Oil-Derived---	
4610	Paraho/SOHIO DFM	0.03 ± 0.005
4801	Geokinetics/Suntech Df-2	0.09 ± 0.013
	---Petroleum-Derived---	
9101	Phillips Reference DF-2, Lot C-345	0.08 ± 0.04
1910	Phillips Reference DF-2, Lot C-747	0.05
1914	DOD Referee DF-2	0.19 ± 0.01
DF-2-1	Ft. Carson DIO DF-2	0.84 ± 0.10
975	API No. 2 Fuel Oil	0.04
976	API Lt. Cat. Cr. Naphtha	<0.002
	---Coal Liquids-Derived---	
978	H-Coal Home Heating Oil	0.8
936	H-Coal Reformed Naphtha	1.4
	---Tar Sands-Derived---	
9527	Suncor Railway DF	0.10 ± 0.02
	---Tar Sands/Petroleum Co-Processing---	
9523	Canadian 1990 DF	4.2 ± 0.1

Table 10. Comparison of 4- to 6-Ring PAH Dermal Tumorigens in Diesel Fuels

Concentration, μg/g

	Petroleum		Shale Oil	Tar Sands	Tar Sands/Petroleum
	1910	1914	4801	9527	9523
PAH	Phillips Reference	DOD Referee	Geokinetics/Suntech	Suncor	1990 DF
Benz(a)anthracene	0.20	1.3	0.29	0.34	26
Chrysene	0.99	1.5	0.80	0.61	147
Benzo(b/j)fluoranthenes	0.26	0.13	0.07	0.08	5.6
Benzo(k)fluoranthene	0.01	0.02	0.03	0.02	2.1
Benzo(a)fluoranthene	0.01	0.04	0.02	0.08	1.2
Benzo(e)pyrene	0.07	0.13	0.06	0.12	6.6
Benzo(a)pyrene	0.03	0.11	0.04	0.04	3.4
Dibenz(a,j)anthracene	0.01	0.01	0.01	0.01	1.7
Indeno[1,2,3-cd]pyrene	0.01	0.02	0.02	0.02	1.7
Dibenz(a,c/a,h)anthracenes	0.01	0.02	0.02	0.01	0.6
Benzo(ghi)perylene	0.01	0.03	0.5	0.02	2.0
Sum	1.61	3.31	1.86	1.35	198

Table 11. Comparison of Tumor Incidence and Indicators of Aromatics Content

Sample No.	Fuel	Ratio by GC		FIA[a]	BaP	5-6	Cumulative Tumor Incidence at Weeks for Assay			
		Aro./Sat.	2MeNaP C13	Aromatics, Vol. %	by HPLC[b], ug/g	Ring PAH[c], ug/g	Complete Tumor. 26	Complete Tumor. 52	Tumor Promotion 26	Tumor Promotion 52
9523	1990 DF	0.35	1.57	67.3	4.2	25	88	94	94	96
1914	DOD Referee DF-2	0.26	0.67	-[d]	0.19	0.51	20	56	92	94
1910	Phillips Reference DF-2	0.19	0.66	28.0	0.05	0.42	18	42	80	94
9527	Suncor Railway DF	<0.16	<0.51	-[d]	0.10	0.40	14	44	72	82
4801	Geokinetics/Suntech DF-2	0.04	0.08	17.8	0.09	0.77	8	38	48	86
975	API No. 2 Fuel Oil	0.07	0.26	21.0	0.04	0.2	2	34	12	48
978	H-Coal Home Ht. Oil	-[d]	"0"[e]	(18.5)[f]	0.8	24	2	18	20	88
976	API Lt. Cat. Cr. Nap.	0.9	"0"[e]	20.3	<0.002	<0.1	0	12	10	36
936	H-Coal Ref. Nap.	1.7	"0"[e]	58.4	1.4	22	0	10	24	46

[a]Fluorescent indicator assay, see Table 1 and Reference (5).
[b]Measurement of benzo(a)pyrene by HPLC.
[c]Sum of benzo[b/j]fluoranthenes, benzo(k)fluoranthene, benzo(a)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, dibenz[a,j]anthracene, indeno[1,2,3-cd]pyrene, dibenz[a,c/a,h]anthracenes, and benzo[ghi]fluoranthene.
[d]Not determined.
[e]2-Methylnapthalene not detected.
[f]Data for non-devolatilized precursor sample.

(Table 4). The most important observation from these data is that the complete tumorigenicity generally parallels the BaP and PAH concentration, except for the H-Coal-derived fuels. The highest concentrations of BaP and total 5- and 6-ring PAH dermal tumorigens were found in the two H-Coal-derived fuels. These were orders of magnitude higher than in all the other fuels, except for the No. 9523 1990 DF. In contrast to this high PAH content, the H-Coal-derived fuels (and particularly the No. 936 Reformed Naphtha) exhibited relatively low complete tumorigenicity and tumor promotion activity. On the other hand, the No. 9523 1990 DF exhibited relatively high activity in both the complete tumorigenicity and tumor promotion assays. The remaining fuels had low PAH content, intermediate tumor promoting activity, and intermediate complete tumorigenicity. These results suggest that the dermal tumorigen PAH are major contributors to the complete tumorigenicity of these fuels, but tumor promotion also is important to the expression of PAH tumorigenicity. Low tumor promoting activity apparently can offset the expected effects of relatively high PAH content, as for the H-Coal fuels. This is one possible explanation for the imperfect agreement between the BaP concentration (a popular "indicator" of potential tumorigenicity) and the complete tumorigenicity of the fuels.

Comparison of Inhalable Volatiles from Fuels

The overall amounts and composition of the inhalable volatiles from the fuels were compared to determine if differences existed which could affect their relative inhalation toxicity to personnel exposed to fuel vapors. The total volatiles were estimated by a gravimetric procedure consisting of allowing ca. 2 mL of fuel to evaporate from an open-topped 24 mL vial which was thermostatted at 25°C in a water bath. As shown in Figure 8, fuel weight loss was most rapid during the first 75 hrs, and slowly reached ca. 10 percent for the Phillips Reference DF-2 over a period of ca. 900 hrs. A period of 75 hrs was chosen as a practical point of comparison. The data in Table 12 indicate that ca. 2 wt. percent of the fuels was evaporated during this period, and that there were no large differences among the DF tested. The volatile matter in the Paraho/SOHIO DFM was in the lowest concentration, while that in the Ft. Carson DF-2 from the DIO was the greatest, but differences were less than a factor of two from the other fuels.

For a more detailed chemical comparison of the inhalable fuel vapors, saturated headspace volatiles accumulating over the liquid fuels inside a closed container were analyzed using capillary column GC, as described elsewhere (15). The saturated vapor represents the air contamination which might be encountered immediately around a fuel spill or from a fuel tank vent or other source of fresh fuel at ca 25°C. Two mL of DF were pipetted into a 24 mL vial, which was sealed with a septum-cap and placed in a water bath thermostatted at 25°C.

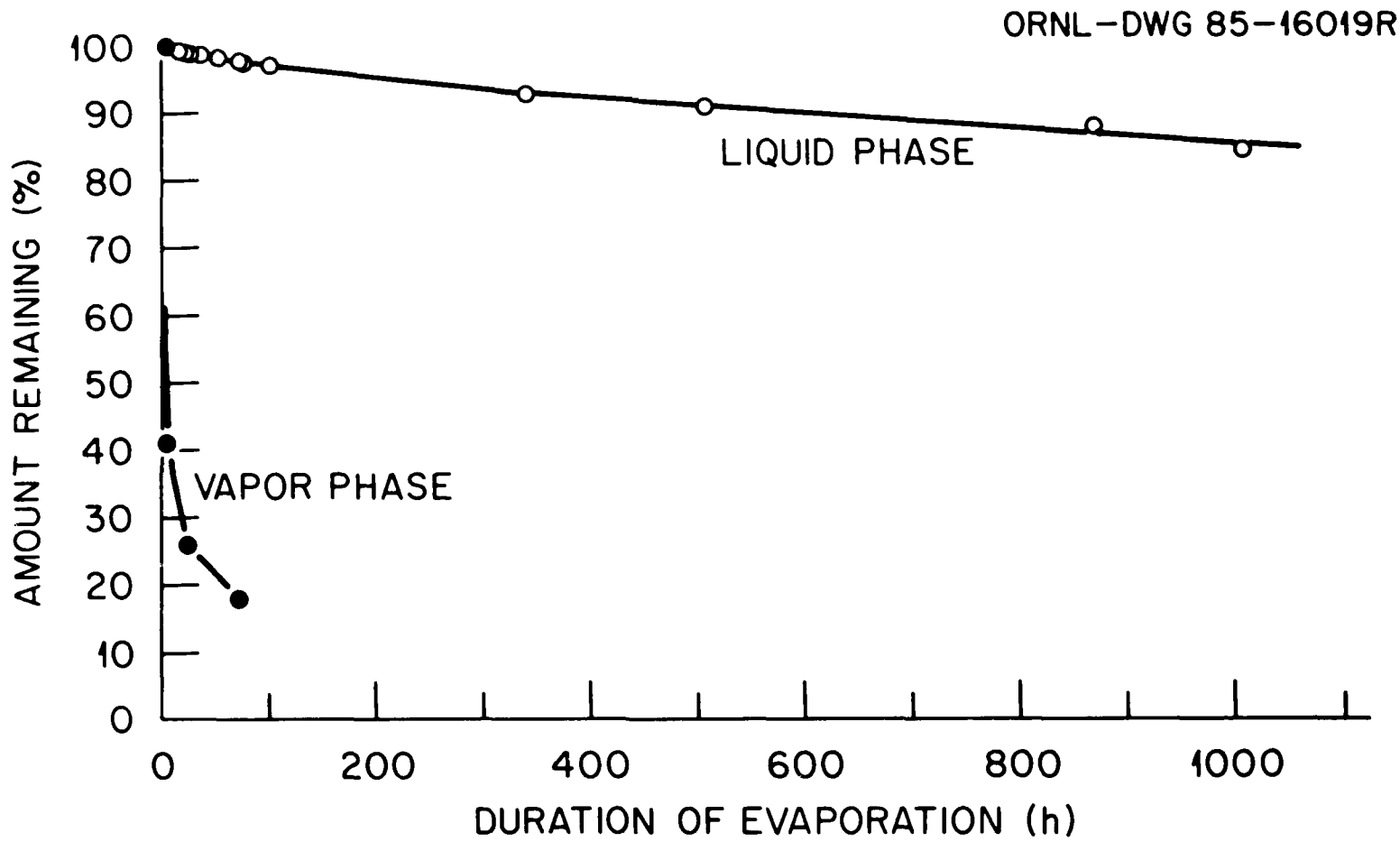

Figure 12. Decreases in Diesel Fuel Organic Liquid and Vapor Phase Masses with Time from Evaporation at Room Temperature

Table 12

Comparison of Inhalable Volatile Matter in Fuels

Sample No.	Fuel	Volatile Matter[a], wt.%
	---Petroleum-Derived---	
1910	Phillips Reference DF-2	2.3
1910	DOD Referee DF-2	2.0
DF-2-1	Ft. Carson DIO DF-2	3.5
4616	WPAFB DFM	2.2
	---Shale Oil-Derived---	
4801	Geokinetics/Suntech DF-2	2.9
4610	Paraho/SOHIO DFM	1.5

[a] Estimated from weight loss of fuel in open container at 25°C for 75 hours.

After a 1.5 hr equilibration period, a 0.5 mL aliquot of headspace vapor was withdrawn by syringe and injected via a no. 3352 Carle valve into a Perkin-Elmer Sigma II GC equipped with a 60 m x 0.32 mm ID x 1 μm film DB-1 bonded phase fused silica column, a column effluent splitter, a flame ionization detector (FID), and a flame photometric detector (FPD) (sulfur mode), and an HP-3390A recording integrator. The FID/FPD split was 60/40 (vol./vol.). The injected vapors were cryogenically focused at the head of the column and were separated by temperature programming from 25°C (hold isothermally 10 min) to 200°C at 2°C/min. The helium carrier gas flow rate was 1.5 mL/min. The inlet and detectors were maintained at 50°C and 250°C, respectively. Quantitation was achieved by the method of external standards using authentic standards prepared in solution and directly injected onto the column via syringe.

Figure 13 compares the capillary column GC resolution of the major organic compounds in the inhalable volatiles of three DF. Only the FID chromatogram is shown. No compounds were detected with the FPD, which was not operating at optimum sensitivity during this work. Therefore, the FPD chromatograms are not shown. Chromatograms for additional fuels are included in the Appendix. All the fuel vapors were found to contain aliphatic hydrocarbons ranging from C_4 through at least C_{10} and alkylated aromatics. These compounds represent the most volatile portion of the DF. Compositional differences were noted among the vapors of the fuels. The vapors of the petroleum-derived DF were somewhat more complex than those of the shale oil-derived DF, particularly in the C_4 and C_5 region. These differences most likely correspond to a greater content of branched and partially unsaturated hydrocarbons in the petroleum DF-2. The tar sands/petroleum coprocessing DF was similar in its simplicity to the shale-oil-derived DF in the C_4-C_6 region, but showed a complexity more like that of the petroleum-derived DF-2 above C_6.

Quantitatively, the concentrations of most major organic compounds in the vapors (Table 13) were similar for the fuels and were in agreement with the relative results for the total volatiles (Table 12). The vapors from the Ft. Carson DF-2 from the DIO exhibited the highest concentrations, while the lowest were found in the vapors from the shale oil-derived DF. In these saturated headspace vapors, concentrations of individual constituents ranged from ca. 6 to nearly 1,000 mg/m^3. The 2-methylbutane was noticeably lower in the vapors of the shale oil fuels. The toluene was very concentrated in the Geokinetics/Suntech DF-2 vapors, which probably reflects the higher content of toluene in the liquid fuel itself (Table 8). These results suggest that differences in the inhalation toxicity among these fuels are likely not to be great, but rather more subtle in nature.

The composition of the vapors from a fuel spill or other source is expected to differ as a function of the temperature of the fuel, because the vapor pressures of the individual compounds in the fuel are temperature-dependent. The vapor composition also is time-dependent because the composition of the liquid fuel will change as the more volatile components are lost by evaporation, the mole fractions of the remaining compounds are changed, and as a result, their partial vapor pressures change (Raoult's law). The influence of fuel temperature is demonstrated by the chromatograms of the fuel vapors shown in Figure 14. Samples of the headspace vapors over sealed vials of no. 1910 Phillips Reference DF-2 were taken at temperatures ranging from 25°C to 65°C and were analyzed by GC as described above. The concentrations of all components increased considerably as the fuel temperature was increased, but the increases were not the same for each component. For example, benzene increased from 16 $\mu g/L$ at 25°C to ca. 62 $\mu g/L$ at 65°C (ca. 4-fold increase), while toluene increased from 35 to 240 $\mu g/L$ (ca. 7-fold), and n-decane rose from 53 to 890 $\mu g/L$ (ca. 17-fold). The effects of temperature on vapor composition probably are not

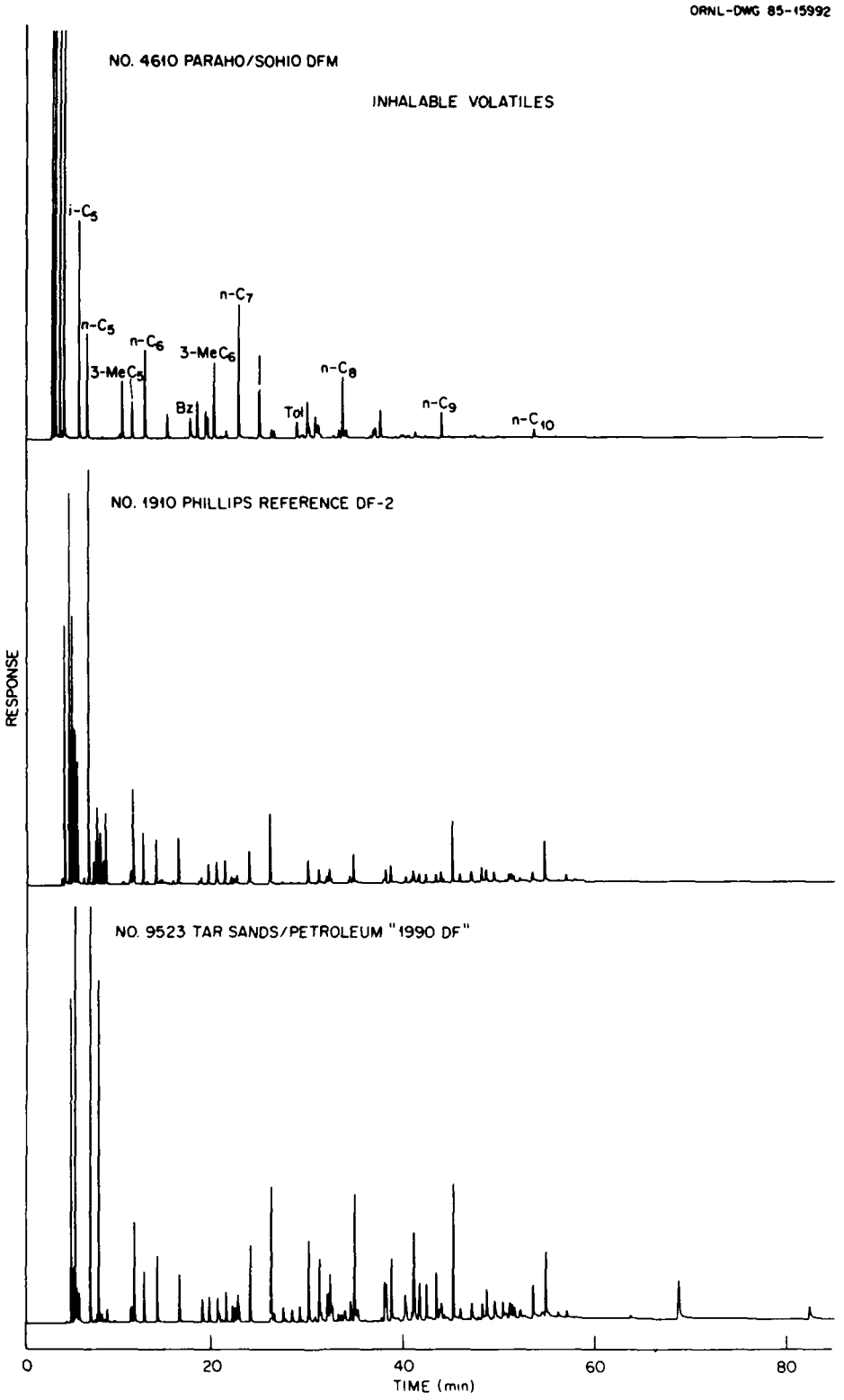

Figure 13. Comparison of the Major Organic Compounds in the Inhalable Volatiles from Diesel Fuels Derived from Shale Oil, Petroleum, and Tar Sands/Petroleum Coprocessing (60 m X 0.32 mm ID x 1.0 μm film of DB-1, temperature programmed from 25°C [hold isothermally 10 min.] to 200°C at 2°C/min.)

Table 13

Comparison of Inhalable Organic Compounds in Headspace Vapors of Diesel Fuels Refined from Petroleum and Shale Oil

Compound	Concentration in Headspace Vapors[a], µg/L					
	Petroleum				Shale Oil	
	No. 1910 Phillips Reference DF-2	No. 1914 DOD Referee DF-2	DF-2-1 Ft. Carson DIO DF-2	No. 4616 WPAFB DFM	No. 4801 Geokinetics-Suntech DF-2	No. 4610 Paraho-SOHIO DFM
2-Methylbutane	260	520	440	920	ND	150
n-Pentane	61	190	260	450	ND	76
2,2-Dimethyl Butane	ND	8	5	13	ND	6
3-Methyl Pentane	53	79	89	110	ND	41
n-Hexane	53	99	190	160	ND	95
Benzene	16	62	33	50	17	29
3-Methyl Hexane	34	59	85	66	11	92
n-Heptane	42	87	170	80	22	148
Toluene	35	140	110	45	970	30
n-Octane	35	69	140	53	70	74
m+p-Xylenes	31	61	80	30	26	6
n-Nonane	74	45	140	45	93	38
1,3,5-Trimethyl Benzene	23	ND	33	ND	22	8
n-Decane	53	12	120	25	57	19

[a]ND = not detected

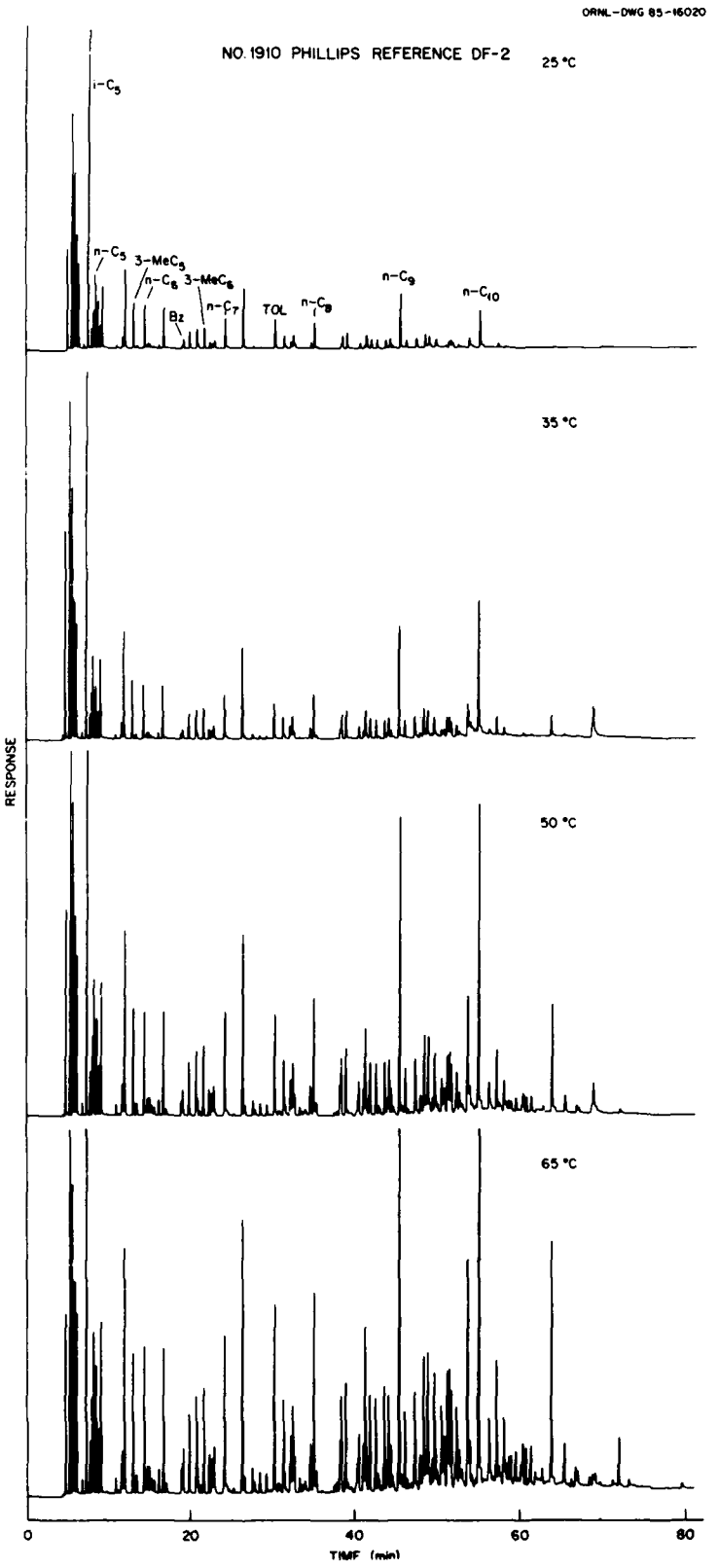

Figure 14. The Influence of Fuel Temperature on the Composition of Inhalable Volatiles from No. 1910 Phillips Reference DF-2 (For GC conditions, see Figure 13.)

quantitatively predictable from the vapor pressure curves of the pure liquids because the relatively high concentrations and large numbers of components do not constitute a system from which ideal behavior can be expected.

As the more volatile compounds in a liquid fuel spill are depleted by evaporation, the composition of the vapor also changes. These changes are illustrated by the chromatograms in Figure 15, which are from the GC analyses of the fuel vapors taken above a sample of no. 1910 Phillips Reference DF-2 at intervals over 73 hours at room temperature (26-27°C). The chromatograms show that the more volatile compounds show considerable depletion even within one hr of evaporation. The C_4 and C_5 hydrocarbons are greatly depleted within one hr and are absent from the vapors by four hrs. By 73 hrs only compounds with boiling points equal to or greater than that of n-nonane (151°C) remain in the vapors. The concentrations of the compounds in the vapors from an actual fuel spill or other source would depend upon a variety of factors which are beyond the scope of this investigation. They would include factors such as the volume of fuel spilled, the rate of leakage, the temperature and ventillation rate, and the porosity of the medium receiving the spill.

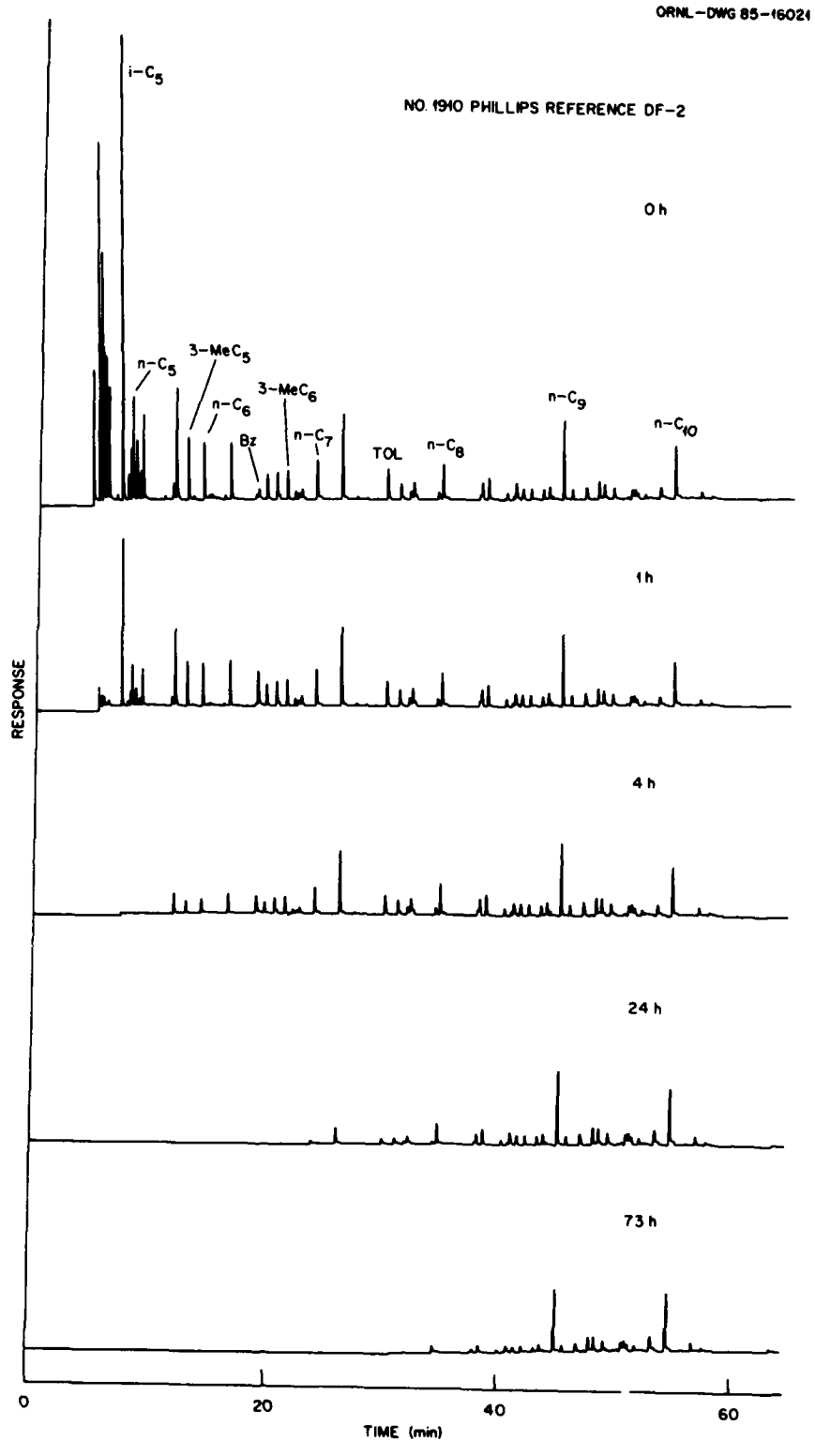

Figure 15. Changes in the Major Organic Compound Composition of Inhalable Volatiles as a Function of Evaporation Time at 25°C for No. 1910 Phillips Reference DF-2 (For GC conditions, see Figure 13.)

CONCLUSIONS

The major conclusions from this work are as follows:

(a) The skin-painting bioassay of highly refined fuels requires long-term (52 weeks to lifetime) applications of neat (100 percent concentration) fuel to the experimental animal in order to achieve measurable responses.

(b) DF derived from petroleum, shale oil, tar sands, tar sands/petroleum co-processing, and coal liquids exhibit both promoting activity and complete tumorigenicity. Promoting activity appears important to the expression of the complete tumorigenicity in such highly refined fuels.

(c) With the exception of the experimental tar sands/petroleum coprocessing 1990 DF, the complete tumorigenicities of the alternate or synthetic fuels are similar to or less than those of the analogous petroleum fuels. The high tumorigenicity of the tar sands/petroleum coprocessing DF appears to result, at least in part, from its high concentrations of PAH dermal tumorigens. The PAH content may be reduced by decreasing the blending ratio of petroleum-derived light cycle oil.

(d) Compositional differences among the bulk liquid fuels and also among their inhalable vapors are mainly quantitative.

(e) Finished, highly refined DF from alternate or synthetic fuels technologies are not likely, with the possible exception of tar sands/petroleum coprocessing, to present a significantly greater toxicological hazard to military personnel than current petroleum-derived DF. Rather, differences in toxicity are likely to be subtle.

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APPENDIX: FUEL TOXICOLOGY PROTOCOL REVIEW

Introduction

The USABRDL is concerned with determining potential toxicological consequences of a changeover of military mobility fuel sources from petroleum to synthetic or alternate. Shale oil, followed by tar sands, is currently considered as a prime candidate as an alternate fuel source. The experimental protocol or protocols which would be best utilized in the comparative toxicity testing of crude and refined mobility fuels derived from petroleum and synthetic or alternate sources are at present not clear. A variety of combinations of animal models, dosing protocols, and other variables have been reported in the literature, and many combinations are possible.

It is the purpose of this review to aid the USABRDL in designing future toxicological tests of mobility fuels. The experimental protocols used in previous studies of crude, upgraded, and refined fuels from natural and synthetic sources are presented, and brief summaries are made of pertinent experimental observations. Part I concerns dermal tumorigenicity studies conducted at ORNL. Part II presents 15 representative experimental protocols conducted at outside Laboratories.

I. Dermal Tumorigenicity Studies at ORNL

Tables A-1 and A-2 present details of experimental protocols and a summary of the percentages of mice developing tumors in dermal tumorigenicity studies conducted at ORNL and one outside lab. Included is protocol no. 5, from studies at Los Alamos National Laboratory, because of the same samples and a very similar protocol to those used at ORNL.

Crude and Upgraded Petroleum and Synthetic Fuels:

Table A-1 presents the experimental protocols used for crude and upgraded petroleum and petroleum substitutes, arranged by study set. Except for protocol no. 8, these are protocols for complete tumorigenicity testing. Protocol no. 8 is a test of tumor initiating activity, in that the sample was applied as an initiator for an extended period of time, followed by a rest, and then a series of doses of tetradecanoyl phorbol acetate (TPA), a classical tumor promoter. In contrast, for complete tumorigenicity testing, only the sample is applied. It acts as both initiator and promoter.

Strain: In these protocols, the C3Hf/Bd strain of mice has been used almost exclusively and a considerable body of data has been generated.

Table A-1. ORNL Protocols for the Mouse Dermal Tumorigenicity Assay of Crude and Upgraded Petroleum and Synthetic Fuels

Protocol No.	Type of Mice	No. of Mice/Group Female	Male	Total	Applications per week	Dose of Application(μL)	Application Duration	Sample	DOE Repository No.	Concentration (w/v %)	Solvent	% of Mice with Skin Tumors Female	Male	Total	Ref.
										100	-	88	76	82	A1
								H-Coal Blend-AWW	931	50	Acetone	96	84	90	A1
										25	Acetone	92	84	88	A1
No. 1	C3Hf/Bd	25	25	50	3	50	life-time								
										100	-	12	16	14	A1
								H-Coal Blend-HDT/L	934	50	Acetone	12	12	12	A1
										25	Acetone	4	0	2	A1
										100	-	20	20	20	A1
								H-Coal Blend-HDT/H	935	50	Acetone	24	44	34	A1
										25	Acetone	16	16	16	A1
										100	-	84	88	86	A1,4
								SRC-II Blend-AWW	916	50	Acetone	28	28	28	A1,4
										25	Acetone	24	16	20	A1,4
										100	-	0	8	4	A1,4
								SRC-II Blend-HDT/L	917	50	Acetone	8	0	4	A1,4
										25	Acetone	4	0	2	A1,4
										100	-	0	0	0	A1,4
								SRC-II Blend-HDT/M	918	50	Acetone	0	0	0	A1,4
No. 2	C3Hf/Bd	25	25	50	3	50	50 weeks			25	Acetone	0	4	2	A1,4
										100	-	0	0	0	A1,4
								SRC-II Blend-HDT/H	919	50	Acetone	0	0	0	A1,4
										25	Acetone	0	0	0	A1,4
										100	-	0	0	0	A5
								H-Coal Pilot Plant Naphtha (Run No. 7)	587	50	Acetone	0	0	0	A5
										25	Acetone	0	0	0	A5
										100	-	0	0	0	A5
								H-Coal Pilot Plant Light Oil (Run No. 7)	589	50	Acetone	0	0	0	A5
										25	Acetone	0	0	0	A5
										100	-	100	100	100	A5
								H-Coal Pilot Plant Heavy Oil (Run No. 7)	591	50	Acetone	92	96	84	A5
										25	Acetone	28	84	56	A5

Table A-1. *ORNL Protocols for the Mouse Dermal Tumorigenicity Assay of Crude and Upgraded Petroleum and Synthetic Fuels (Cont'd)*

Protocol No.	Type of Mice	No. of Mice/Group Female	Male	Total	Applications per week	Dose of Application(μL)	Application Duration	Sample	DOE Repository No.	Concentration (w/v %)	Solvent	% of Mice with Skin Tumors Female	Male	Total	Ref
										100	-	0	0	0	A5
								H-Coal Pilot Plant Light Oil (Run No. 8)	887	50	Acetone	0	0	0	A5
										25	Acetone	0	0	0	A5
										100	-	100	84	92	A5
								H-Coal Pilot Plant Heavy Oil (Run No. 8)	888	50	Acetone	100	92	96	A5
										25	Acetone	76	60	68	A5
										50	7/3:Acetone Cyclohexane	50	40	45	A6
								H-Coal Raw Distillate	1601	25	"	0	70	35	A6
										12.5	"	10	10	10	A6
										6.25	"	0	0	0	A6
										50	"	10	0	5	A6
								H-Coal Raw Distillate - HDT/L	1602	25	"	0	0	0	A6
										12.5	"	10	0	5	A6
No. 3	C3Hf/Bd	10	10	20	3	50	65 weeks			6.25	"	0	0	0	A6
										50	"	0	0	0	A6
								H-Coal Raw Distillate - HDT/M	1603	25	"	0	0	0	A6
										12.5	"	0	0	0	A6
										6.25	"	0	0	0	A6
										50	"	10	0	5	A6
								H-Coal Raw Distillate - HDT/H	1604	25	"	0	0	0	A6
										12.5	"	0	0	0	A6
										6.25	"	0	10	5	A6
										50	"	0	30	15	A6
								Recluse Petroleum		25	"	0	0	0	A6
										12.5	"	0	0	0	A6
										6.25	"	0	0	0	A6
								Paraho Shale Oil	4601	50	Cyclohexane	87	100	94	A2,7
No. 4	C3Hf/Bd	15	15	30	3	50	40 weeks	Hydrotreated Paraho Shale Oil	4602	50	Cyclohexane	33	87	50	A2,7
								Hydrotreated Shale Oil Residue	4607	50	Cyclohexane	53	100	77	A2,7

Table A-1. ORNL Protocols for the Mouse Dermal Tumorigenicity Assay of Crude and Upgraded Petroleum and Synthetic Fuels (Cont'd)

Protocol No.	Type of Mice	No. of Mice/Group Female	Male	Total	Applications per week	Dose of Application(μL)	Application Duration	Sample	DOE Repository No.	Concentration (w/v %)	Solvent	% of Mice with Skin Tumors Female	Male	Total	Ref.
										80	7/3:Acetone Cyclohexane	-	-	30	A8
								Paraho Shale Oil	4601	40	"	-	-	35	A8
										10	"	-	-	25	A8
No. 5	C3Hf/Bd	20	20	40	3	50	33 weeks								
										80	"	-	-	5	A8
								Hydrotreated Paraho Shale Oil	4602	40	"	-	-	0	A8
										10	"	-	-	2.5	A8
										80	"	-	-	30	A8
								Occidental Shale Oil		40	"	-	-	15	A8
										10	"	-	-	17.5	A8
										25	"	48	72	60	A9
No. 6	C3Hf/Bd	25	25	50	3	50	32 weeks	Coal Gasifier ESP Tar	UMD-83	12.5	"	100	100	100	A9
										6.25	"	88	92	90	A9
								Synthoil	1202	2	3:7/Acetone Cyclohexane	-	-	92	A10
										0.7	"	-	-	28	A10
										0.5	"	-	-	8	A10
										0.01	"	-	-	4	A10
								COED Oil	1106	1.6	"	-	-	8	A10
										0.7	"	-	-	4	A10
										0.34	"	-	-	2	A10
										0.07	"	-	-	2	A10
No. 7	C3Hf/Bd	25	25	50	3	50	24 months								
								LETC Shale Oil	4101	5	"	-	-	90	A10
										1	"	-	-	2	A10
										0.6	"	-	-	2	A10
										0.2	"	-	-	0	A10
								Composite Petroleum	5107	4	"	-	-	8	A10
										0.8	"	-	-	0	A10
										0.6	"	-	-	0	A10
										0.16	"	-	-	0	A10

Table A-1. ORNL Protocols for the Mouse Dermal Tumorigenicity Assay of Crude and Upgraded Petroleum and Synthetic Fuels (Cont'd)

Protocol No.	Type of Mice	No. of Mice/Group Female	Male	Total	Applications per week	Dose of Application(uL)	Application Duration	Sample	DOE Repository No.	Concentration (w/v %)	Solvent	% of Mice with Skin Tumors Female	Male	Total	Ref.
								H-Coal VSO, Crude	1310	6	Acetone	-	-	65	All
								H-Coal VSO, Neutral Fraction	-	6	Acetone	-	-	55	All
								H-Coal VSO, Aliphatics	-	6	Acetone	-	-	0	All
								H-Coal VSO, PAH Fraction	-	6	Acetone	-	-	90	All
								H-Coal VSO, Neutral Polar	-	6	Acetone	-	-	25	All
No. 8	C3Hf/Bd	-	-	20	3	50	53 weeks*	SRC-II, FOB	1701	6	Acetone	-	-	30	All
								SRC-II, FOB, Neutral Fraction	-	6	Acetone	-	-	20	All
								SRC-II, FOB, Aliphatics	-	6	Acetone	-	-	0	All
								SRC-II, FOB, PAH Fraction	-	6	Acetone	-	-	85	All
								SRC-II, FOB, Neutral Polar	-	6	Acetone	-	-	5	All

*This is a tumor initiating activity protocol. The sample was applied for 20 weeks. After a 3 week rest, TPA was applied at an 8 ug exposure dose, three times per week in acetone, for 30 more weeks.

Table A-2 ORNL Protocols for the Mouse Dermal Tumorigenicity Assay of Highly Refined Petroleum and Synthetic Fuel Products

Sample	DOE Repository No.	Type of Mice	Concentration (w/v %)	Solvent	Mice Applied Female	Mice Applied Male	Mice Applied Total	Applications per week	Dose per Application (µL)	Application Duration	% of Mice with Skin Tumors Female	% of Mice with Skin Tumors Male	% of Mice with Skin Tumors Total	Ref	Pro
Refined Petroleum and Synthetic Petroleum Products															
Coal Derived Products															
H-Coal Naphtha Reformate	936	C3Hf/Bd	100	-	25	25	50	3	50	life-time	0	0	0	A1	1
			50	Acetone	25	25	50	3	50	"	0	0	0	A1	1
			25	Acetone	25	25	50	3	50	"	4	4	4	A1	1
H-Coal Home Heating Oil	978	C3Hf/Bd	100	-	25	25	50	3	50	life-time	20	36	28	A1	1
			50	Acetone	25	25	50	3	50	"	20	28	24	A1	1
			25	Acetone	25	25	50	3	50	"	16	16	16	A1	1
Oil Shale Derived Products															
JP-5, Jet Fuel	4608	C3Hf/Bd	100	-	15	15	30	3	50	40 weeks	20	7	13	A2	2
			50	Cyclohexane	15	15	30	3	50	"	27	20	24	A2	2
JP-8, Jet Fuel	4609	C3Hf/Bd	100	-	15	15	30	3	50	40 weeks	13	20	17	A2	2
			50	Cyclohexane	15	15	30	3	50	"	13	7	10	A2	2
DFM, Diesel Fuel Marine	4610	C3Hf/Bd	100	-	15	15	30	3	50	40 weeks	13	0	7	A2	2
			50	Cyclohexane	15	15	30	3	50	"	0	7	4	A2	2
Petroleum Derived Products															
API Lt. Cat. Cr. Naphtha	978	C3Hf/Bd	100	-	25	25	50	3	50	life-time	12	0	6	A1	1
			50	Acetone	25	25	50	3	50	"	4	12	8	A1	1
			25	Acetone	25	25	50	3	50	"	16	16	16	A1	1
API Petr. No. 2 Fuel Oil	975	C3Hf/Bd	100	-	25	25	50	3	50	life-time	4	20	12	A1	1
			50	Acetone	25	25	50	3	50	"	12	16	14	A1	1
			25	Acetone	25	25	50	3	50	"	4	4	4	A1	1
Phillips Reference No. 2 Diesel Fuel	9101	SENCAR	100	-	20	20	40	1	200	38 weeks	0	0	0	A3	9
			10	Cyclohexane	20	20	40	1	200	"	-	-	2.5	A3	9
			1	Cyclohexane	20	20	40	1	200	"	0	0	0	A3	9
JP-5, Jet Fuel	4614	C3Hf/Bd	100	-	15	15	30	3	50	40 weeks	0	0	0	A2	2
			50	Cyclohexane	15	15	30	3	50	"	7	0	4	A2	2
JP-8, Jet Fuel	4615	C3Hf/Bd	100	-	15	15	30	3	50	40 weeks	0	13	7	A2	2
			50	Cyclohexane	15	15	30	3	50	"	7	0	4	A2	2
DFM, Diesel Fuel Marine	4610	C3Hf/Bd	100	-	15	15	30	3	50	40 weeks	7	7	7	A2	2
			50	Cyclohexane	15	15	30	3	50	"	0	7	4	A2	2
Phillips Reference No. 2 Diesel Fuel	9101	SENCAR	100	-	20	20	40	2	200	38 weeks	-	-	60	A3	10
			10	Cyclohexane	20	20	40	2	200	"	-	-	10	A3	10
			1	Cyclohexane	20	20	40	2	200	"	-	-	5	A3	10

Dose groups consist of 10 to 25 male and female mice per group, except for protocol no. 8, where a random group of 20 mice (including both sexes) was used per dose level.

Dosing: In all cases a sample volume of 50 μL was applied to the shaved dorsal skin (shaved two days before initiation and ca. weekly thereafter) of the animals three times a week. A comparison with twice-weekly dosing was reported in references (A2), (A7), and (A10). Samples were applied to groups of mice in doses generally varying by serial factors of two (e.g., 100%, 50%, 25%, 12.5%, and 6.25%). In some protocols, (e.g., no. 7) four dose groups were used. This allows a wide dosage range to be studied. In other protocols, (e.g., no. 8 or 4) only one dose level was applied. Although this protocol does not provide a dose-response evaluation, it does allow a more economical comparison of samples and provides valuable input for the design of more definitive bioassay protocols.

Acetone, acetone/cyclohexane (3/7 or 7/3, v/v), or cyclohexane alone have been used as solvents. Of these solvents, acetone has been used most frequently in recent studies. It causes minimum skin irritation and has no detectable tumorigenic response. Dilutions with cyclohexane have been used to improve solubility characteristics for some samples.

Duration: The duration of these studies ranged from 32 weeks to lifetime. The latter depends upon the lifetime of the particular strain of animals used and their response to the test agents. Generally, ca. 24 months (ca. 104 weeks) would be typical for a lifetime study with C3H mice if the test agent is not strongly tumorigenic or toxic. For some highly refined samples (see following discussion), 28 or 30 months may be required before all animals have expired or developed tumors.

Results: A brief summary of the observations from the studies listed in Table A-1 follows:

(1) Comparing crude (unrefined) materials from different sources, it is confirmed that dermal tumorigenicity decreases in the general order coal > shale > petroleum.

(2) Even "low severity" catalytic hydrotreatment ("HDT/L", generally corresponding to a 50% reduction in the total nitrogen content of the sample) drastically reduces the tumorigenicity of coal liquids to levels comparable to that of crude petroleum.

(3) The tumorigenicity of shale oil is reduced, but not eliminated by hydrotreatment.

(4) The tumorigenicity of crude coal liquids is contributed mainly by the polycyclic aromatic hydrocarbon subfraction of the neutral chemical fraction.

Finished Petroleum and Synthetic Fuel Products:

Protocols and observations for dermal tumorigenicity studies of refined fuels are displayed in Table A-2. The refined fuels include reformed naphthas, jet and diesel fuels, and home heating oil/no. 2 fuel oils, which are arranged by source in the table. Note that the comparative studies (indicated by the common reference or protocol numbers) cut across the sample source groups in Table A-2 and also across the crude and upgraded samples in Table A-1. Except for protocol no. 10 (comparison of promoting activity), these are tests of complete tumorigenicity. The promoting activity protocol differs from complete tumorigenicity testing mainly in that a single dose of an initiating agent (typically, 7,12-dimethylbenz[a]anthracene) is applied to the animals prior to repeated doses of the sample.

Strain: As with the crude and upgraded samples, the C3H/Bd strain has been used most often. For two protocols, the SENCAR ("SENsitive to CARcinogenicity") strain was used. This latter strain is being used currently in tumor promotion studies comparing diesel fuels derived from shale oil, petroleum, tar sands, and tar sands/petroleum co-processing for USABRDL. The same protocol is employed in DOE/Office of Fossil Energy-sponsored tumor promotion studies of naphthas and home heating oils/no. 2 fuel oils derived from coal liquids and petroleum. Each dose group consisted of equal numbers (15 to 25) of mice from both sexes, for a total of 30 to 50 mice per group.

Dosing: All C3H/Bd mice were dosed three times per week with 50 μL of sample. References (A2) and (A7) also describe a protocol with two doses applied per week. However, the SENCAR mice were dosed with 200 μL once per week in the complete tumorigenesis protocol and twice per week following a single tumor initiator dose of 2.52 μg of 7,12-dimethylbenz(a)anthracene in the tumor promotion protocol. The SENCAR mouse is larger than the C3H/Bd mouse, and a larger volume of sample can be applied. The larger dose with the SENCAR mice also does not require that the mice be shaved, whereas the C3H must be shaved ca. weekly during the experiment. However, both strains are shaved two days before initiation. The doses for the C3Hf/Bd mice consist of neat (100%) sample and 50% and 25% dilutions in acetone or cyclohexane, while in the SENCAR strain, doses of neat (100%), 10%, and 1% (both of the latter in acetone) were used.

Duration: The complete carcinogenicity studies with the C3Hf/Bd mice were carried out for periods of 40 weeks to lifetime. With highly refined fuels such as the no. 936 H-Coal Reformed Naphtha, tumorigenicity is at or below the limit of detection of the protocol,

and a few animals may survive through 28 or 29 months. A routine protocol of 38 weeks was used for the complete carcinogenicity and tumor promotion assays involving the SENCAR strain. This time duration can be extended. The protocol for current USABRDL and DOE/FE-sponsored tumor promotion studies of refined fuels is scheduled for 52 weeks with the SENCAR strain.

Results: A summary of the observations made in the studies listed in Tables A-1 and A-2 is as follows:

(1) The extensive upgrading and refining conducted upon the fuels greatly decreases, and in some cases almost eliminates, the tumorigenicity which was exhibited by the crude fuels.

(2) Small differences in complete tumorigenicity are observed between fuel products, i.e., the shale jet fuels appear slightly more tumorigenic than the shale diesel fuel, and the coal or petroleum home heating oils/no. 2 fuel oils are at least as tumorigenic or more tumorigenic than the reformed naphthas.

(3) Small differences in complete tumorigenicity are observed between fuels derived from different sources. The coal-derived home heating oil is more potent than is the petroleum no. 2 fuel oil, and the shale-derived jet fuels are slightly more tumorigenic than are the petroleum-derived jet fuels.

(4) Tumor promoting activity was found in a petroleum-derived no. 2 diesel fuel.

Comments

The dermal tumorigenicity studies at ORNL which would be of the most interest to USABRDL are mainly those for the refined fuels. The results suggest that complete tumorigenicity studies should be conducted on a lifetime duration in order to have sufficient sensitivity and discrimination power to detect and resolve the small differences expected in the low tumorigenicity of highly refined mobility fuels. Either the C3H or SENCAR strain would be applicable; however, the greater sensitivity to carcinogenesis of the latter suggests it would be advantageous. The results of this study, described elsewhere in this report, indicate that tumor promoting activity also is important to the tumorigenicity of diesel fuels. The SENCAR strain is highly useful for promotion assays.

II. Representative Protocols Reported in the Literature for Mouse Dermal Tumorigenicity Assays

In the last sixty years, a large number of experimental protocols for mouse dermal tumorigenicity assays has been reported. The fifteen protocols presented in Table A-3 have been taken from the literature and are representative protocols in terms of their historical backgrounds or their features. The names assigned to these protocols are directly derived from the laboratories or agencies which carried out the experiments. Those agencies are: Oak Ridge National Laboratory (ORNL), Pacific Northwest Laboratory (PNL), Los Alamos National (Scientific) Laboratory (LANL), Argonne National Laboratory (ANL), Laboratories of the British Manchester Committee on Cancer, Institute of Experimental and Clinical Medicine (Tallinn, Estonia, S.S.R.), Kettering Laboratory (University of Cincinnati), Carnegie-Mellon, Exxon, and International Research and Development Corporation (Mattawan, MI). Protocols Nos. 16-19 have more than one agency name listed. The names of these protocols are arranged such that the first name assigned to the protocol is that laboratory which actually carried out the experimental work.

The most important and useful information describing the tumorigenicity of test materials is the complete tumorigenicity data. Thus, major protocols No. 9 to No. 21 discussed in this study are complete tumorigenicity protocols. In these protocols only the test material (neat or diluted) is applied to the animals. It acts as both initiator and promoter. Protocol No. 22 is a tumor promotion protocol, in which the animals are initiated with a single dose of 7,12-dimethylbenz(a)anthracene (DMBA) two days after shaving. Seven days later, the neat test materials are applied twice a week for 52 weeks. Protocol No. 23 is a protocol for tumor initiation; in that test, material (diluted with acetone) is applied as an initiator. Two weeks later, phorbol myristate acetate (PMA or TPA) is applied to the initiated area twice weekly for a period of six months. The advantage of using the tumor initiation test is that it reduces the test duration time and in most cases the assay still generates sufficient information for predicting the complete tumorigenicity of those test materials. Similarly, in a short test time (38 weeks or one year), a tumor promotion assay is able to reveal the potential complete tumorigenicity of a test material which contains only a trace amount of tumor initiators.

In the following, animal models, dosing protocols, and other variables of those protocols are described and evaluated.

Strain: In these thirteen complete tumorigenicity protocols (No. 9 to No. 21), the C3H strain (including C3Hf/Bd, C3H/Bd, C3Hf/He, and C3H/HeJ) of mice has been used the most often, and a considerable body of data has been generated and reported. Other strains such as white mice, SKH, and CD-1 were utilized in some studies. The SENCAR

Table A-3. Representative Protocols Reported in the Literture for the Mouse Dermal Tumorigenicity Assay of Crude and Upgraded Petroleum and Synthetic Fuels

Protocol No.	Name of Protocol	Strain of Mice	No. of Mice/Group			Applications per Week	Dose per Application (µL)	Application Duration	Sample Description	Concentration (W/V%)	Solvent	% of Mice with Skin Tumor			Ref. (year)
			Female	Male	Total							Female	Male	Total	
									H-Coal Naphtha Reformate	100	-	0	0	0	A1
									DOE Rep. No. 936	50	Acetone	0	0	0	(1986)
										25	Acetone	4	4	4	
									H-Coal Home Heating Oil	100	-	20	36	28	A1
									DOE Rep. No. 978	50	Acetone	20	28	24	(1986)
										25	Acetone	16	16	16	
									API Lt. Cat. Cr. Naphtha	100	-	12	0	6	A1
									DOE Rep. No. 976	50	Acetone	4	12	8	(1986)
										25	Acetone	16	16	16	
									API Petr. No. 2 Fuel Oil	100	-	4	20	12	A1
No. 9	DOE-CRWL -1	C3Hf/Bd	24	25	50	3	50	Life-time	DOE Rep. No. 975	50	Acetone	12	16	14	(1986)
										25	Acetone	4	4	4	
									H-Coal Blend-A&W	100	-	88	76	82	A1
									DOE Rep. No. 931	50	Acetone	96	84	90	(1986)
										25	Acetone	92	84	88	
									H-Coal Blend-HDT/L	100	1	12	16	14	A1
									DOE Rep. No. 934	50	Acetone	12	12	12	(1986)
										25	Acetone	4	0	2	
									H-Coal Blend-HDT/H	100	-	20	20	20	A1
									DOE Rep. No. 935	50	Acetone	24	44	34	(1986)
										25	Acetone	16	16	16	

Table A-3. Representative Protocols Reported in the Literature for the Mouse Dermal Tumorigenicity Assay of Crude and Upgraded Petroleum and Synthetic Fuels (Cont'd)

Protocol No.	Name of Protocol	Strain of Mice	No. of Mice/Group			Applications per Week	Dose per Application (μL)	Application Duration	Sample Description	Concentration (W/V%)	Solvent	% of Mice with Skin Tumor			Ref. (year)
			Female	Male	Total							Female	Male	Total	
									Wilmington Crude Petroleum	50	Acetone	-	-	100	A12
										5	Acetone	-	-	46	(1981)
										0.5	Acetone	-	-	0	
No. 10	DOE-PNL -1	C3Hf/Bd	25	25	50	3	50	2 yrs	Livermore Shale Oil	50	Acetone	-	-	100	A12
										5	Acetone	-	-	100	(1981)
										0.5	Acetone	-	-	0	
									SRC-II Light Distillate	50	Acetone	-	-	0	A12
										5	Acetone	-	-	0	(1981)
										0.5	Acetone	-	-	0	
									SRC-II Heavy Distillate	50	Acetone	-	-	100	A12
										5	Acetone	-	-	100	(1981)
										0.5	Acetone	-	-	82	
									Paraho Shale Oil	10	Acetone/Cyclohexane (7/3)	-	-	76	A8,13 (1981,1984)
No. 11	DOE-LANL	C3Hf/Be	-	-	40	3	50	100 weeks	Hydrotreated Paraho Shale Oil	10	Acetone/Cyclohexane (7/3)	-	-	68	A8,13 (1981,1984)
									Louisiana Crude Petroleum	10	Acetone/Cyclohexane (7/3)	-	-	92	A8,13 (1981,1984)

Table A-3. Representative Protocols Reported in the Literature for the Mouse Dermal Tumorigenicity Assay of Crude and Upgraded Petroleum and Synthetic Fuels (Cont'd)

Protocol No.	Name of Protocol	Strain of Mice	No. of Mice/Group			Applications per Week	Dose per Application (µL)	Application Duration	Sample Description	Concentration (W/V%)	Solvent	% of Mice with Skin Tumor			Ref. (year)
			Female	Male	Total							Female	Male	Total	
									HYGAS Recycle Oil	-	Acetone	-	-	91	A14 (1986)
No. 12	DOE-AML	SKH	25	25	50	3	25-50 mg	52 weeks	UNDERC Tar	-	Acetone	-	-	93	A14 (1986)
									Refined Pennsylvanian Petroleum	100	-	-	-	2	A15 (1928)
No. 13	Laboratories of British Manchester Committee on Cancer	White Mice	-	-	100	2	a brushful	60 weeks	Refined Californian Petroleum	100	-	-	-	8	A15 (1928)
									Refined Texas Petroleum	100	-	-	-	0	A15 (1928)
									Shale-Derived Fuel Oil	100	-	-	-	13	A16 (1979)
No. 14	Institute of Experimental and Clinical Medicine, Tallinn (Estonia, S.S.R.)	White Mice	-	-	~100	2	-	25 weeks	Shale-Derived Impregnating Oil	100	-	-	-	9	A16 (1979)
									Shale-Derived Chamber Oven Tar	100	-	-	-	45	A16 (1979)
									Petroleum Paraffinic Mineral Oil	100	-	-	0	0	A17 (1965)
No. 15	Kettering Laboratory	C3H/HeJ	-	30	30	2	50	80 weeks	Petroleum Naphthenic Mineral Oil	100	-	-	0	0	A17 (1965)

Table A-3. Representative Protocols Reported in the Literature for the Mouse Dermal Tumorigenicity Assay of Crude and Upgraded Petroleum and Synthetic Fuels (Cont'd)

Protocol No.	Name of Protocol	Strain of Mice	No of Mice/Group			Applications per Week	Dose per Application (μL)	Application Duration	Sample Description	Concentration (W/V%)	Solvent	% of Mice with Skin Tumor			Ref. (year)
			Female	Male	Total							Female	Male	Total	
No. 18	Kettering Laboratory, OSHA	C3H	-	20-30	20-30	2-3	50	-	Shale Oil Sample #1 (Heat transfer process)	100	-	-	90	90	A18 (1979)
									Shale Oil Sample #2 (Heat transfer process)	100	-	-	63	63	A18 (1979)
									Shale Oil Sample #3 (Retort Combustion process)	100	-	-	67	67	A18 (1979)
									Petroleum Crude Oil (Texas)	100	-	-	0	0	A18 (1979)
									Petroleum Crude Oil (Asphaltic)	100	-	-	0	0	A18 (1979)
									Petroleum Paraffinic Distillate (Uncracked Crude)	100	-	-	95	95	A18 (1979)
									Industrial Fuel Oil Residuum (Catalytically Cracked)	100	-	-	100	100	A18 (1979)

Table A-3. Representative Protocols Reported in the Literature for the Mouse Dermal Tumorigenicity Assay of Crude and Upgraded Petroleum and Synthetic Fuels (Cont'd)

Protocol No.	Name of Protocol	Strain of Mice	No. of Mice/Group			Applications per Week	Dose per Application (µL)	Application Duration	Sample Description	Concentration (W/VX)	Solvent	% of Mice with Skin Tumor			Ref. (year)
			Female	Male	Total							Female	Male	Total	
	Kettering Laboratory, Exxon, Sun Tech, API, Texaco Diamond Shamrock, Chevron								South Lou. Whole Crude	100	-	-	30	30	A19,20 (1984,1985)
No. 17		C3H/HeJ	-	50	50	2	50	18 months	Sout Lou. Light Straight Run Naphtha	100	-	-	21	21	A19,20 (1984,1985)
									South Lou. Straight Run Kerosine	100	-	-	30	30	A19,20 (1984,1985)
									South Lou. Straight Run Gas Oil	100	-	-	34	34	A19,20 (1984,1985)
									South Lou. Heavy Vacuum Gas Oil	100	-	-	81	81	A19,20 (1984,1985)
									Sout Lou. Vacuum Residum	100	-	-	0	0	A19,20 (1984,1985)
									Kuwait Whole Crude	100	-	-	56	56	A19,20 (1984,1985)
									Kuwait Light Ends	100	-	-	0	0	A19,20 (1984,1985)
									Kuwait Light Straight Run Naphtha	100	-	-	25	25	A19,20 (1984,1985)
									Kuwait Straight Run Kerosine	100	-	-	15	15	A19,20 (1984,1985)
									Kuwait Straight Run Gas Oil	100	-	-	3	3	A19,20 (1984,1985)
									Kuwait Heavy Vacuum Gas Oil	100	-	-	91	91	A19,20 (1984,1985)
									Kuwait Vacuum Residue	100	-	-	2	2	A19,20 (1984,1985)

Table A-3. Representative Protocols Reported in the Literature for the Mouse Dermal Tumorigenicity Assay of Crude and Upgraded Petroleum and Synthetic Fuels (Cont'd)

Protocol No.	Name of Protocol	Strain of Mice	No. of Mice/Group			Applications per Week	Dose per Application (μL)	Application Duration	Sample Description	Concentration (W/V%)	Solvent	% of Mice with Skin Tumor			Ref. (year)
			Female	Male	Total							Female	Male	Total	
No. 18	Kettering Laboratory, API, TOSCO	C3H	-	-	-	2	50	120	Raw Shale Oil	100	-	-	-	92	A13 (1984)
									Hydrotreated Shale Oil (0.33% H)	100	-	-	-	54	A13 (1984)
									Hydrotreated Shale Oil (0.25% H)	100	-	-	-	10	A13 (1984)
									South Louisiana Crude	100	-	-	-	20	A13 (1984)
									Kuwait Crude	100	-	-	-	38	A13 (1984)
No. 19	Carnegie-Mellon, Union Carbide	C3H	-	30	30	3	a brushful	12 months	Coal-Derived Middle Oil Stream	100	-	-	46	46	A21 (1960)
									Coal-Derived Light Oil Stream Residue	100	-	-	66	66	A21 (1960)
									Coal-Derived Pasting Oil	100	-	-	94	94	A21 (1960)

Table A-3. Representative Protocols Reported in the Literature for the Mouse Dermal Tumorigenicity Assay of Crude and Upgraded Petroleum and Synthetic Fuels (Cont'd)

Protocol No.	Name of Protocol	Strain of Mice	No. of Mice/Group			Applications per Week	Dose per Application (µL)	Application Duration	Sample Description	Concentration (W/V%)	Solvent	% of Mice with Skin Tumor			Ref. (year)
			Female	Male	Total							Female	Male	Total	
									Tar Sands Bitumen:						
									Untreated Naphtha	100	-	-	2	2	A22 (1986)
									Light Gas Oil	100	-	-	30	30	A22 (1986)
No. 20	Exxon	C3H/HeJ	-	50	50	3	25	Life-time	Heavy Gas Oil	100	-	-	96	96	A22 (1986)
									Synthetic Crude Oil	100	-	-	28	28	A22 (1986)
									Petroleum Intermediate Catalytically Cracked Distillate:						
									< 338 C	100	-	-	4	4	A22 (1986)
									338-371 C	100	-	-	22	22	A22 (1986)
									> 371 C	100	-	-	82	82	A22 (1986)
									SRC First Stage Middle Distillate	~ 8	PEG 400	0	0	0	A23 (1987)
										~ 4	PEG 400	0	0	0	A23 (1987)
										~ 1	PEG 400	0	0	0	A23 (1987)
No. 21	International Research and Development Corporation, Mattawan, MI	CD-1	65	65	130	3	~ 40	2 years							
									TSL Second Stage Middle Distillate	~ 8	PEG 400	0	0	0	A24 (1987)
										~ 4	PEG 400	0	0	0	A24 (1987)
										~ 1	PEG 400	0	2	1	A24 (1987)

Table A-3. Representative Protocols Reported in the Literature for the Mouse Dermal Tumorigenicity Assay of Crude and Upgraded Petroleum and Synthetic Fuels (Cont'd)

Protocol No.	Name of Protocol	Strain of Mice	No. of Mice/Group			Applications per Week	Dose per Application (μL)	Application Duration	Sample Description	Concentration (W/V%)	Solvent	% of Mice with Skin Tumor			Ref. (year)
			Female	Male	Total							Female	Male	Total	
									H-Coal Naphtha Reformate DOE Rep. No. 936	100	-	4	52	28	A25 (1987)
									H-Coal Home Heating Oil DOE Rep. No. 978	100		92	72	82	A25 (1987)
									API Lt. Cat. Cr. Naphtha DOE Rep. No. 976	100	-	20	60	40	A25 (1987)
No. 22	DOE-ORNL -2	Sencar	25	25	50	2 (promotion protocol)	200	52 weeks	API Petr. No. 2 Fuel Oil DOE Rep. No. 975	100	-	80	100	90	A25 (1987)
									Phillips Reference DF-2 DOE Rep. No. 1910	100	-	92	96	94	A25 (1987)
									DOD Referee DF-2 DOE Rep. No. 1914	100	-	92	96	94	A25 (1987)
									1990 Tar Sands/Pet. DF DOE Rep. No. 9523	100	-	92	100	96	A25 (1987)
									SUNCOR Tr. Sd. Rail. DF DOE Rep. No. 9527	100	-	92	72	82	A25 (1987)
									Geo./Sun. Shale DF-2 DOE Rep. No. 4601	100	-	80	92	86	A25 (1987)

Table A-3. Representative Protocols Reported in the Literature for the Mouse Dermal Tumorigenicity Assay of Crude and Upgraded Petroleum and Synthetic Fuels (Cont'd)

Protocol No.	Name of Protocol	Strain of Mice	No. of Mice/Group			Applications per Week	Dose per Application (µL)	Application Duration	Sample Description	Concentration (W/V%)	Solvent	% of Mice with Skin Tumor			Ref. (year)
			Female	Male	Total							Female	Male	Total	
									SRC-I 550-600 F Distillate	50	Acetone	28	-	28	A26 (1983)
									SRC-I 600-650 F Distillate	50	Acetone	10	-	10	A26 (1983)
									SRC-I 650-700 F Distillate	50	Acetone	15	-	15	A26 (1983)
No. 23	DOE-CRUL -2	CD-1	-	30	30	Initiation protocol	50	With 2X5 ug per week of PMA for 6 months	SRC-I 700-750 F Distillate	50	Acetone	40	-	40	A26 (1983)
									SRC-I 750-800 F Distillate	50	Acetone	75	-	75	A26 (1983)
									SRC-I 800-850 F Distillate	50	Acetone	92	-	92	A26 (1983)
									SRC-II 850-900 F Distillate	50	Acetone	63	-	63	A26 (1983)

("SENsitive to CARcinogenicity") strain was used in the tumor promotion protocol (protocol No. 22) and the CD-1 strain was applied in the tumor initiation study (protocol No. 23).

No. of Mice/Group: In protocol No. 9, No. 10, No. 12, No. 21, and No. 22, dose groups consist of 25 to 65 male and female mice per group. For protocol No. 11, No. 13, and No. 14, a random group of 40 or 100 mice (including both sexes) were used per dose level. In protocol No. 15, No. 16, No. 17, No. 19, No. 20, and No. 23, the dose group only consists of male mice (20 to 50 per group). The number of mice per dose group for protocol No. 18 was not mentioned in the literature. Obviously, the choice of number and sex of mice is very inconsistent. However, the protocols with 25 male and female mice per dose group may be more optimal since the tumorigenicity response to each sex is often reported to be different and the tumorigenicity data for 50 mice (total) per dose group is sufficient to describe the tumorigenicity of most test materials.

Dose and Number of Applications: In these complete tumorigenicity protocols, a sample volume (neat or diluted) of 50 μL has been often used. The application of a "brushful" dose (in protocols No. 13 and No. 19) is clearly not a quantitative method. Since the SENCAR mouse is larger than other strains of mice (such as C3Hf/Bd), a larger volume (200 μL) of test material was used in the tumor promotion protocol (protocol No. 22). In all cases a test material was applied to the shaved dorsal skin of the animal two or three times a week. That means that both two and three times per week application protocols are appropriate.

Application Duration: The duration of these complete tumorigenicity protocols ranged from 25 weeks to lifetime. The lifespan of C3H mice is about 30 months. The tumorigenicity response of test materials is recognized to have a direct correlation with the activities and concentrations of tumorigens in those samples. That means, if the test sample is a very strong tumorigen (such as high-boiling range fractions of crude coal-derived oils), six months would be a sufficient time to develop tumors. If the test sample is highly refined, then a lifetime period of application is needed in order to describe a very low tumor incidence. Based on the usefulness and completeness of tumorigenicity testing, a lifetime test may be a necessary approach for detecting any potentially tumorigenic fossil fuel materials, especially highly refined mobility fuels.

Sample Concentration and Solvent: Samples were applied to groups of mice in doses generally varying by factors of 2 or 10 (e.g., 100%, 50%, and 25%; or 50%, 5%, and 0.5%; or 8%, 4%, and 1%). In many protocols listed in Table A-3, only one dose level (100%) was applied. Since data on the dose-response relationship is needed for determining the limit of the tumorigenic threshold for a sample, a definitive bioassay protocol would require three or four dose levels. Acetone and

acetone/cyclohexane (7/3) have been used as solvents. Acetone has been used the most frequently. It causes minimum skin irritation and has no detectable tumorigenic response. Dilutions with cyclohexane have been useful to improve solubility characteristics for some samples.

Sample Type and Tumorigenicity Result: The main purpose of this study is to evaluate the experimental protocols, therefore, only a few representative fossil fuel materials were chosen for Table A-3. Those test samples cover very broad categories including crude, distilled, and refined materials derived from petroleum, oil shale, coal, or tar sands. Because the protocol variables (such as strain and dosing protocol) and the test materials are so different from protocol to protocol, the tumorigenicity data from these tests cannot be readily compared. However, several important observations can be made.

1. Some test materials derived from petroleum, oil shale, coal, or tar sands can produce very highly tumorigenic responses in the mouse dermal tumorigenicity assay.

2. Despite different fossil fuel origins, the extensive upgrading and refining necessary to produce finished fuel products greatly decreases, and in some cases almost eliminates, the tumorigenicity which was exhibited by the crude fuels. In other words, there is no general indication that finished fuels derived from synthetic or alternate sources are more tumorigenic than those derived from petroleum.

3. Similarly, despite the different fuel origins, high boiling range fractions (> ca. 650°F/343°C) always are more tumorigenic than low boiling range fractions.

Comments

The results of this protocol review indicate that for complete tumorigenicity tests of highly refined fuels, a lifetime bioassay with multiple dose levels is needed. The highest dose should be with the neat (100% concentration) fuel. A candidate protocol can be described as follows: Groups of 25 female and 25 male inbred Specific Pathogen Free C3Hf/Bd mice are assigned to test groups at 10-11 weeks of age. The animals are maintained five per cage. Each material is tested at three doses [100% (neat), 50%, and 25%] by applying 50 μL of the material to the shaved backs of the mice three times per week. Acetone is used as the diluent to prepare the 50% and 25% test dosage. Skin painting continues for the lifetimes of the animals (ca. 26-30 months). An attractive alternate strain is the SENCAR mouse, because of its greater sensitivity to carcinogenesis. Although the volume applied is greater than for the C3H mouse (200 μL vs 50 μL), tumor responses with neat (100% concentration) DF can be substantial within 12 months of treatment.

Either strain appears useful for tumor promotion studies, in which a single initiating dose of DMBA is followed by twice-weekly applications of the fuel for ca. 52 weeks, including a high dose with the neat fuel. The C3H strain requires a greater dose of initiator than does the SENCAR strain (ca. $\leq$ 200 μg vs 2.52 μg DMBA). As for the complete tumorigenicity assay, multiple dose levels are employed to determine the dose-response relationship.

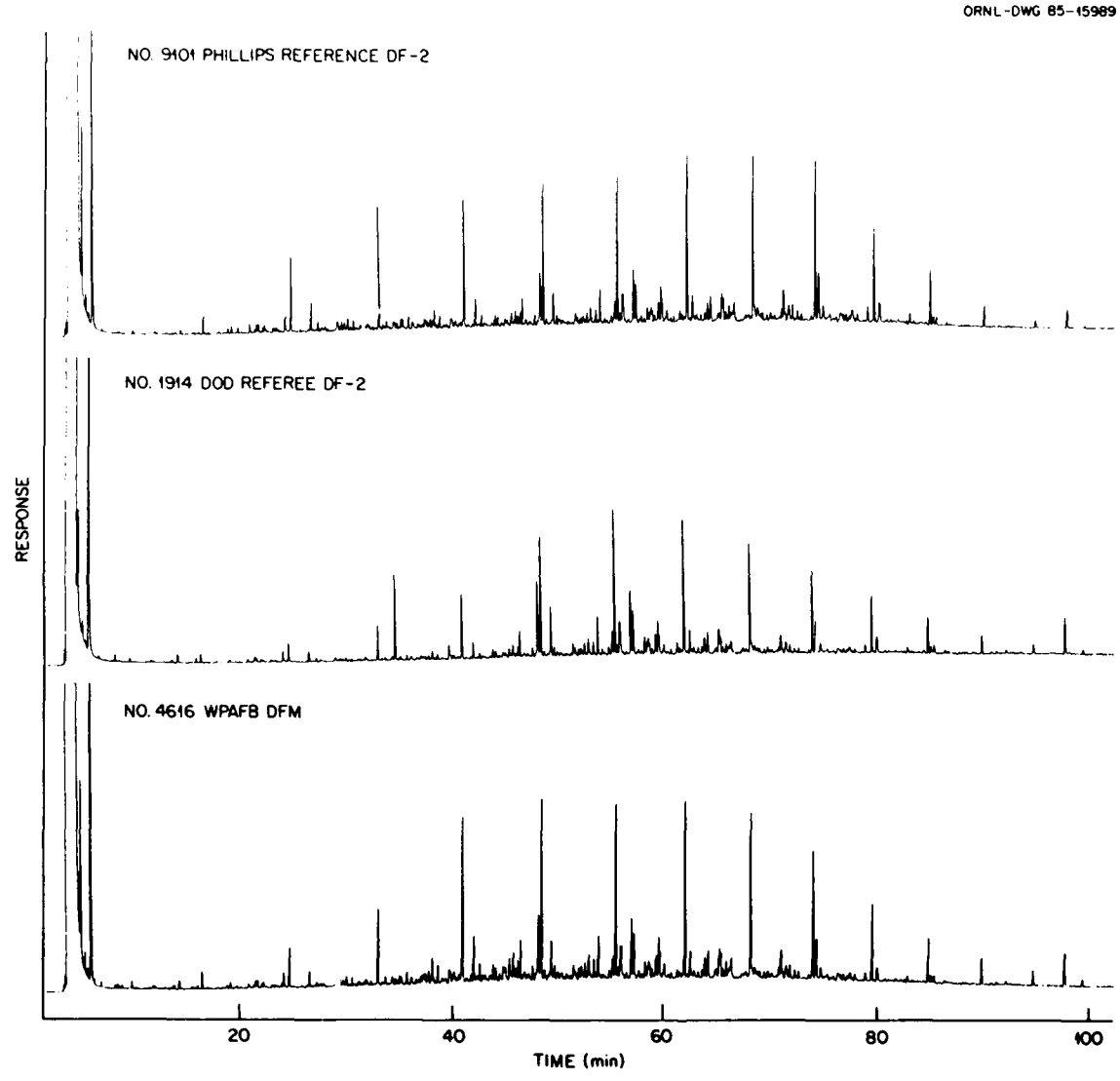

Figure A-1. Comparison of the Major Organic Compounds in Diesel Fuels Derived from Petroleum (For GC conditions, see Figure 10.)

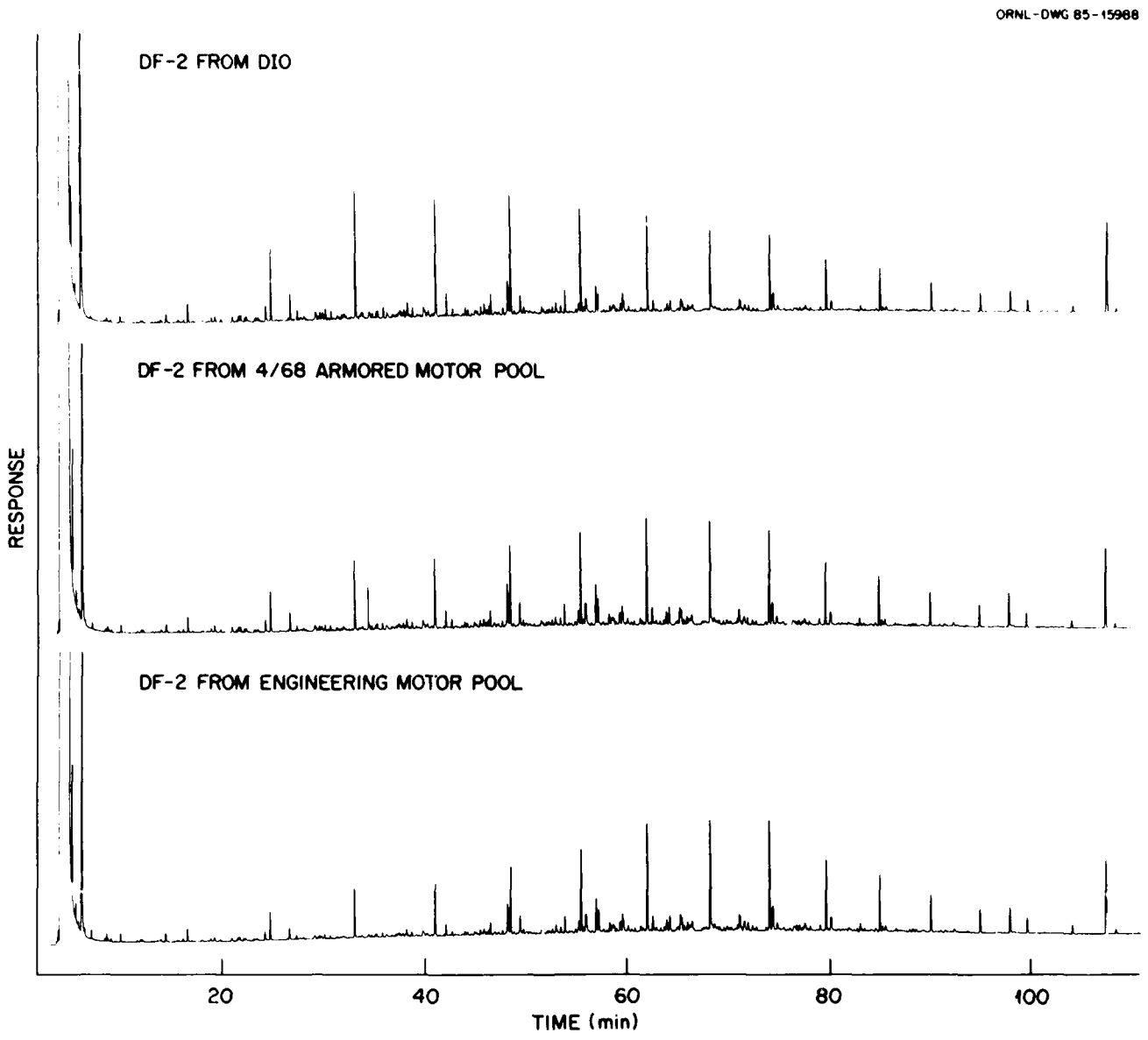

Figure A-2. Comparison of the Major Organic Compounds in DF-2 Collected at Fort Carson Motor Pools (For GC conditions, see Figure 10.)

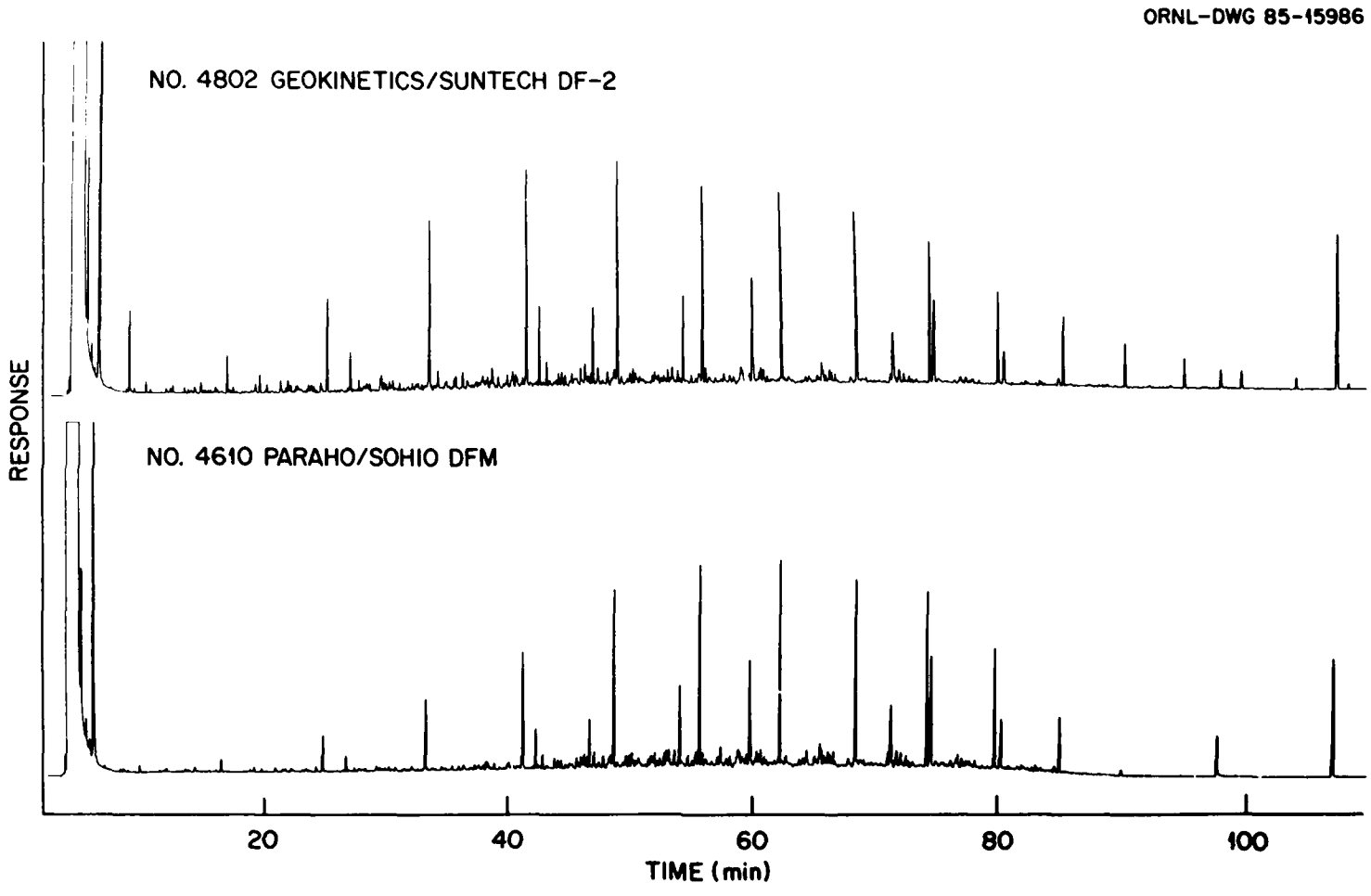

Figure A-3. Comparison of the Major Organic Compounds in Diesel Fuels Derived from Shale Oil (For GC conditions, see Figure 10.)

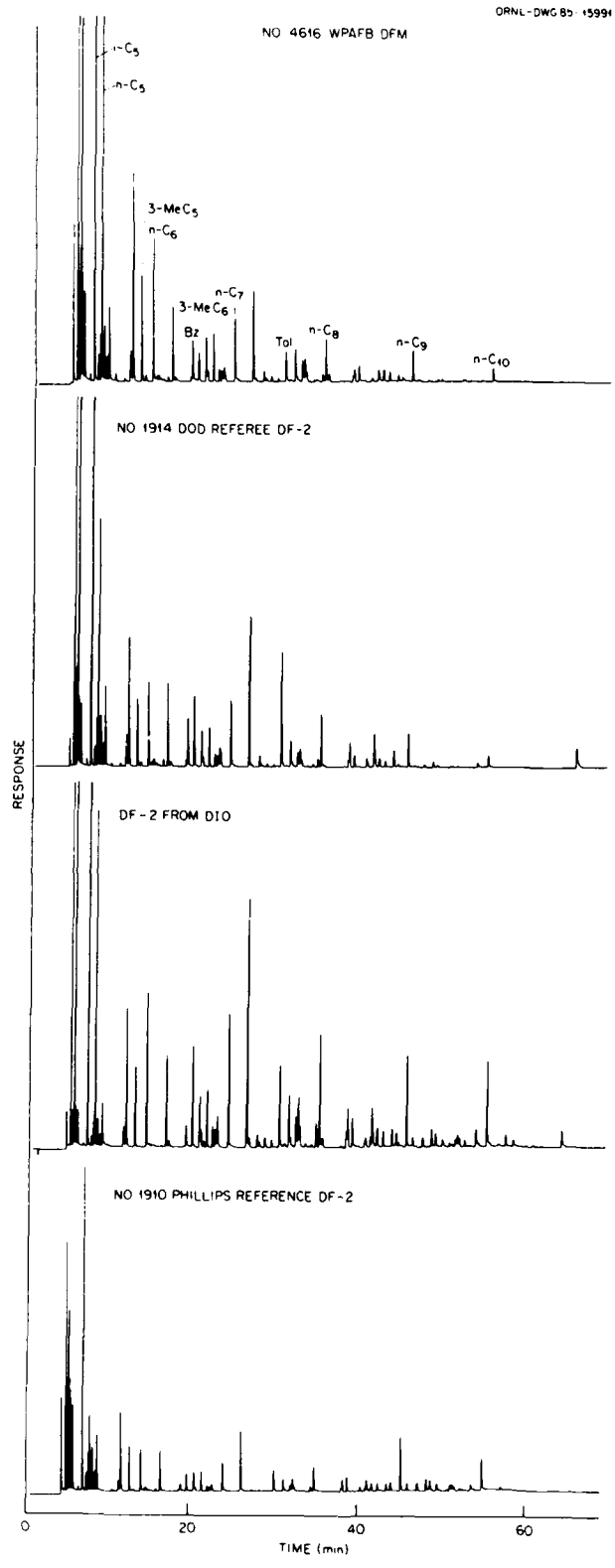

Figure A-4. Comparison of the Major Organic Compounds in the Inhalable Volatiles from Several Petroleum-Derived Diesel Fuels (For GC conditions, see Figure 13.)

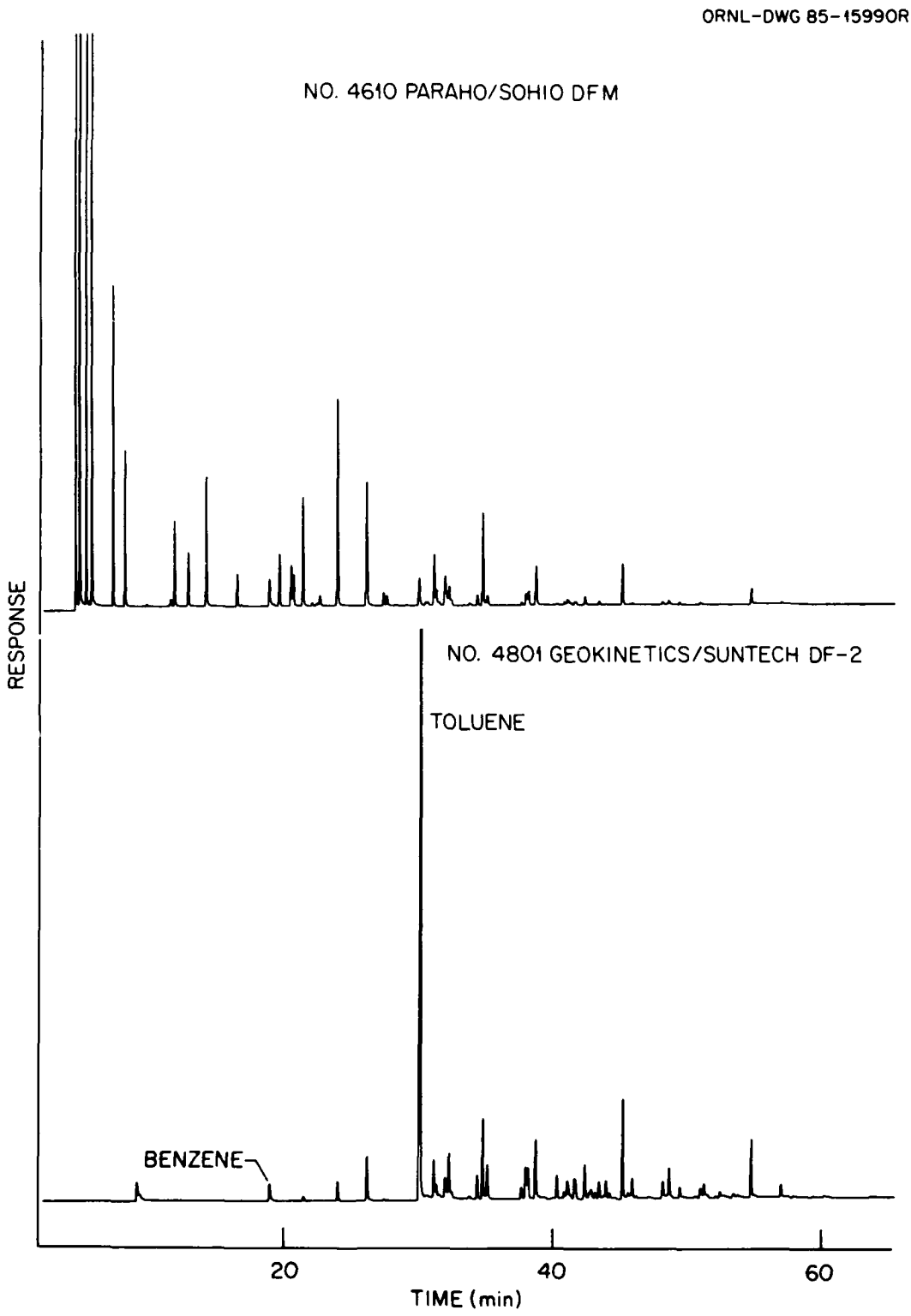

Figure A-5. Comparison of the Major Organic Compounds in the Inhalable Volatiles from Two Shale Oil-Derived Diesel Fuels (For GC conditions, see Figure 13.)

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